

CEREAL CHEMISTRY

Vol. III

May, 1926

No. 3

EFFECT OF SODIUM NITRATE APPLIED AT DIFFERENT STAGES OF GROWTH OF WHEAT ON THE BAKING QUALITY OF THE FLOUR

By JEHIEL DAVIDSON, Bureau of Chemistry
and

J. H. SHOLLENBERGER, Bureau of Agricultural Economics

United States Department of Agriculture

(Received for publication March 9, 1926)

In a series of experiments carried on by the Bureau of Chemistry since 1916 (1917, 1918, 1923), it has been shown that nitrates as well as ammonium salts increase the yield of wheat only when applied to the soil during the vegetative stage, that they increase the protein¹ content and prevent "yellow berry" when applied at the time of heading, and that they have practically no effect when applied at the milk stage. Similar results have been subsequently obtained by Gericke (1920) and by Neidig and Snyder (1922).

An experiment conducted by the Bureau of Chemistry at College Park, Md. (1922), showed that when nitrates were applied to different plots at three successive periods during the vegetative stage, the increase in yield of wheat was directly related to the earliness of application, while the increase in the protein content of the grain was inversely related to the same factor.

More recent experiments, the detailed results of which are as yet unpublished, have shown that even under conditions when an application of sodium nitrate at the vegetative stage does not increase the yield of wheat, the protein content of the grain is nevertheless very materially increased when the application is made at the time of heading.

The effect of the nitrate applications on the chemical character of the wheat proteins has not yet been determined, but the fact that the increase in nitrogen content is accompanied by an in-

¹The term "protein" refers to the total nitrogen as determined by the Kjeldahl method multiplied by the appropriate factor.

crease in sulphur, as brought out by unpublished results, indicates that at least part of the nitrogen increase is in the form of true proteins.

Wheat naturally high in protein generally yields a "strong" flour, which gives a bread of superior qualities. It has seemed desirable, therefore, to ascertain the baking qualities of the flour made from wheat in which the protein content has been increased by the application of sodium nitrate at the time of heading.

Wheat Used in the Baking Tests

The wheat for the baking tests was obtained from an experiment carried out by the Bureau of Chemistry in 1924-25. The experiment consisted of two series: The "solid" series, in which the wheat was drilled in rows 8 inches apart; and the "spaced" series, in which the rows were 24 inches apart. Soft red winter wheat of the variety Purplestraw was used. Each series comprised plots which received sodium nitrate applications at different periods between planting and heading, each plot receiving only one application during the entire growing season. The periods at which applications were made to the plots of the solid series were: Time of planting, time of emergence, early spring, and time of heading. In the spaced series the applications were made at the same dates as in the solid series, as well as at several additional periods between early spring and heading time. The experiment was run in triplicate. The full results will be presented in another publication. Suffice to say here that the yields of grain from the spaced plots were practically equal to those from the solid plots.

Effect of Heading-Time Applications of Sodium Nitrate on Yield and Composition of the Milling Products of Wheat

It was considered worth while, in connection with the baking tests, to get an idea of how the milling products in general were affected by the treatment which results in a high protein content of the grain. Table I gives the yield and general composition of the milling products from two control plots and from two plots which received sodium nitrate at the time of heading. In both cases—the two controls and the two treated plots—one belonged to the solid and the other to the spaced series. There being no essential differences in the yield and composition of their milling

products, the solid and the spaced plots may be considered as duplicating each other with reference to treatment.

As seen from Table I, only the nitrogen content of the milling products was distinctly and consistently affected by the heading-time applications. All three of the milling products share in the nitrogen increment, the flour and the bran having increased about 30 per cent and the shorts about 23 per cent over the controls.

The other determinables vary, but the variations are not in any way consistent either with the fertilizer treatment or with the distance between the rows, except, perhaps, the ash content of the bran and shorts, that of the bran being lower and that of the shorts higher than the controls. The differences involved, however, are slight and may be accidental.

Effect of Heading-Time Application of Sodium Nitrate on Baking Qualities of Wheat

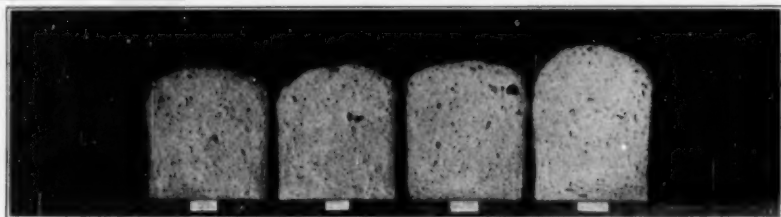
Samples from variously treated plots were put through the complete routine followed in the milling section of the Bureau of Agricultural Economics in making a milling and baking test (1924). The results are given in Table II.

As was to be expected, applications in fall and early spring did not greatly affect the quality and composition of the wheat. In the plan of the experiment these plots served to test the effect of time of application on yield only, and it may be mentioned here incidentally that the spring applications resulted in the highest yields of grain. The rise in protein, correlated with the lateness of application, between fall and early spring, though consistent, is very slight. The increase in protein, however, resulting from the heading-time application is relatively large and leaves no doubt as to tendency. In the spaced series the several applications between spring and heading time resulted in a distinct gradual rise in proteins, corroborating the results of the College Park experiments (1922). The percentages of "vitreous" kernels corroborate the direct correlation between the protein content of the grain and the so-called "vitreousness" or "flintiness" which was shown in the previous experiments of the Bureau of Chemistry (1917, 1918, 1923).

On the other hand, it is shown that there is no correlation between the weight per 1000 kernels and weight per bushel and the protein content. This corroborates results reported in a previous publication (1923), where the question was discussed

fully. Similar results were obtained by Bailey and Hendel (1923) while correlating the weight per 1000 kernels and the protein content of middlings flour for a number of years. The fact that the high protein wheat resulting from the heading applications of nitrogen remains as plump and yields as much flour as the wheat of normal protein content grown under the same environmental conditions, seems to be an important point in favor of this fertilizer treatment.

The other columns in Table II show that practically all the measurements and scorings used in testing the quality of bread are in favor of the wheat from the plots which received applications of sodium nitrate at the time of heading. In the solid series the results are thoroly consistent. In the spaced series the honors are contested between the plot which received the nitrate application at heading time and that which received it just prior to heading.



FROM "SOLID" SERIES
Left to right

1. Control plot
2. Sodium nitrate applied in fall
3. Sodium nitrate applied in spring
4. Sodium nitrate applied at heading time

The photographs of the loaves of bread given here show the effect of the time of application of sodium nitrate on the baking quality of the wheat flour even more clearly than do the data in Table II. In the solid series the loaf from the plot which received sodium nitrate at the time of heading towers above the others, showing at the same time the finest texture. In the spaced series the loaf volume of the breads rises gradually, culminating in the greatest volume for the plot which likewise received the sodium nitrate application at the time of heading.

Mangels (1925) found that the average protein content of North Dakota wheat fluctuated in 1921-24 between 11.33 and 15.02 per cent, and stated that the baking qualities of this wheat vary less than its protein content. He believes, therefore, that the

quality of the gluten in the high protein wheat is inferior to that in the low protein wheat. His results seem to be at variance with those reported in this article. However, it is not difficult to find an explanation for these seemingly contradictory results. Under natural conditions, low and high protein wheats are produced by different environmental conditions. In North Dakota,



FROM "SPACED" SERIES
Left to right

1. Control plot
2. Sodium nitrate applied in fall
3. Sodium nitrate applied in spring
4. Sodium nitrate applied 3 weeks previous to heading time
5. Sodium nitrate applied 2 weeks previous to heading time
6. Sodium nitrate applied at heading time

for instance, as shown by the data compiled by Mangels (*loc. cit.*), high protein wheat is produced under adverse conditions which result in low yields. It is possible, therefore, that the same factors also affect unfavorably the quality of the gluten as well as that of the other constituents of the flour. However, the high protein wheat produced by applying nitrates at the time of heading is raised under the same environmental conditions as the normal wheat. This accounts for the fact that the baking quality of its increased gluten content, so far as can be judged from the baking tests, as well as its other qualities, is not impaired by the treatment which results in its high protein content.

Summary

The nitrogen increment of high protein wheat resulting from the application of sodium nitrate to the soil at the time of heading is distributed among the three milling products—flour, shorts, and bran. This wheat, while being as plump and yielding as much flour as normal wheat raised under the same conditions, gives a bread having superior qualities.

TABLE I
EFFECT OF SODIUM NITRATE APPLIED AT TIME OF HEADING ON THE YIELD AND COMPOSITION OF THE MILLING PRODUCTS OF WHEAT

Time of application of sodium nitrate	Distance between rows in plots	Flour				Bran				Shorts			
		Yield	Moisture*	Ash*	Protein (Nx5.7)	Yield	Moisture*	Ash*	Protein (Nx5.7)	Yield	Moisture*	Ash*	Protein (Nx5.7)
		%	%	%	%	%	%	%	%	%	%	%	%
Control	8 (solid)	73.8	12.95	.45	10.3	16.9	12.92	6.59	13.1	8.7	11.16	3.66	14.5
"	24 (spaced)	73.1	12.74	.49	10.7	18.1	13.38	6.55	13.3	8.1	10.92	4.01	15.4
Heading	8 (solid)	72.0	12.51	.49	13.9	17.7	12.58	6.29	17.2	9.1	10.57	4.13	18.5
"	24 (spaced)	73.4	12.42	.47	13.4	17.3	12.60	6.24	17.2	9.1	10.72	4.20	18.9

* Determined in the Chemical Research Laboratory of the Bureau of Agricultural Economics by the methods described in U. S. Dep Agr Bull. 1187.

TABLE II
EFFECT OF TIME OF APPLICATION OF SODIUM NITRATE ON THE BAKING QUALITIES OF WHEAT

Time of applica- tion of sodium nitrate	Distance between rows in plots	Vitreous		Weight per 1000 kernels	Test weight per bushel, dock free	Flour yield	Water absorp- tion of flour	Volume of loaf	Weight of loaf	Color of crumb	Texture of crumb	Grain crumb	Protein (Nx5.7)†			Ash in	
		in.	%										Wheat	Flour	%	%	%
Control	8 (solid)	70.1	32.2	60.9	70.9	57.9	1920	495	93.0	GS	89.8	11.4	10.2	0.37			
Fall	"	81.1	31.4	61.9	70.4	57.1	1930	492	94.0	GS	90.5	11.5	10.4	0.36			
Spring	"	85.5	29.8	61.0	70.7	59.4	2010	499	92.4	GC	89.0	11.9	10.7	0.36			
Heading	"	96.2	32.6	61.7	69.2	58.2	2190	487	94.8	VG	93.3	14.8	13.5	0.44			
Control	24 (spaced)	78.9	32.8	61.4	70.7	57.2	2030	497	92.1	VG	91.3	12.1	11.0	0.37			
Fall	"	82.9	28.9	60.6	69.1	57.1	2030	496	92.2	VG	91.0	11.8	10.4	0.34			
Spring	"	81.8	29.2	60.0	70.2	57.4	2160	494	91.9	G	90.9	12.5	10.9	0.36			
3 weeks previous to heading	"	86.5	30.3	59.5	70.7	58.5	2270	496	92.3	VG	92.0	13.5	12.5	0.40			
2 weeks previous to heading	"	95.5	31.2	61.1	69.3	60.3	2270	488	93.7	E	93.3	15.5	13.8	0.36			
Heading	"	89.6	31.1	60.5	68.9	61.2	2300	496	92.8	E	93.7	15.1	13.9	0.42			

* E, excellent; VG, very good; G, good; GS, good-solid; GC, good-crumbly.

† Determined in the Chemical Research Laboratory of the Bureau of Agricultural Economics. Basis, 13.5% moisture.

Literature Cited

- Bailey, C. H., and Hendel, Julius.
1923. Correlation of wheat kernel plumpness and protein content. J. Am. Soc. Agron., Vol. 15, pp. 345-350.
- Davidson, Jehiel.
1922. The effect of nitrates applied at different stages of growth on the yield, composition, and quality of wheat. J. Am. Soc. Agron. Vol. 14, pp. 118-122.
- and LeClerc, J. A.
1917-8. The effect of sodium nitrate applied at different stages of growth on yield, composition, and quality of wheat. J. Am. Soc. Agron., Vol. 4, pp. 145-154; and Vol. 5, pp. 193-198.
- 1923. Effect of various inorganic nitrogen compounds applied at different stages of growth on the yield, composition, and quality of wheat. J. Agr. Research, Vol. 23, pp. 55-68.
- Gericke, W. F.
1920. On the protein content of wheat. Science, N. S., Vol. 52, pp. 446-447.
- Mangels, C. H.
1925. Effect of climate and other factors on the protein content of North Dakota wheat. Cereal Chem. Vol. II, pp. 288-297.
- Neidig, R. E., and Snyder, R. S.
1922. The effect of available nitrogen on the protein content and yield of wheat. Idaho Agr. Exp. Sta. Res. Bull. No. 1.
- Shollenberger, J. H., Marshall, W. K., and Coleman, D. A.
1924. Experimental milling and baking. U. S. Dep. Agr. Bull. 1187.

AN IMPROVED METHOD FOR THE PREPARATION OF WHEAT GLIADIN¹

By M. J. BLISH AND R. M. SANDSTEDT

Department of Agricultural Chemistry, University of Nebraska,
Lincoln

(Received for publication February 27, 1926)

During the course of recent studies of certain properties of the gluten proteins of wheat flour, an observation was made which has led to what may reasonably be considered as an improved and simplified method for preparing wheat gliadin in quantity, as well as in a high state of purity. The improvement and simplification of the new method over the established methods (see Osborne and Harris 1906-07 and Dill and Alsberg 1925) involves the elimination of the repeated preliminary extractions with 50 to 70 per cent alcohol, as well as the tedious process of evaporating the alcoholic extracts under reduced pressure, during which process it is necessary to maintain the original alcoholic concentration by frequent additions of strong alcohol. In the new method very little alcohol is required beyond a certain amount of absolute alcohol for reprecipitating and drying the protein.

Wood (1907), Upson and Calvin (1915), and others have observed that in the absence of salts wet crude gluten is readily hydrated and dispersed in very dilute acids, giving an opaque sol. Sharp and Gortner (1922) have determined that maximum hydration occurs at a pH of 3.25 to 2.25. If a salt is added to this sol, the process is reversed, and the gluten settles out as a gel with its original physical properties. Sharp and Gortner (1922) noted that the hydration capacity of wet crude gluten was greatly altered by preliminary drying, even though the drying had been carried on at a temperature as low as 45-50°C. Bailey (1925) calls attention to the fact that "the colloidal behavior of dough can be modified by seemingly simple manipulations," and that wetting (with either water or alcohol) followed by drying at room temperature caused decided reductions in the gas-retaining property of dough from flour thus treated. Sharp and Gortner (1923) consider that of the two gluten proteins, glutenin and gliadin, glutenin is the protein to which gluten owes its susceptibility to those changes in colloidal properties which are so important from the bread-making standpoint.

¹Published with the approval of the Director as Paper No. 23, Journal Series, Nebraska Agricultural Experiment Station.

Experimental

Certain alterations in the physico-chemical properties of gluten, caused by drying under specified conditions, are the basis for the new method for the preparation of gliadin which is to be proposed in this communication. It was found, as is to be expected, that when wet crude gluten was placed in .1N to .01 N acetic acid, the gluten imbibed water rapidly and, after a few hours, with occasional shaking, a very opaque but uniformly dispersed sol was obtained. When a salt such as NaCl or CaCl_2 was added, with stirring, the gluten gathered on the stirring rod, and could thus be recovered, with practically all of its original physical properties, toughness, elasticity, etc.

When the same experiment was attempted with gluten which had been previously dried in a high vacuum at 60 to 65°C., and ground to a powder, the results were decidedly different. Even after prolonged shaking, the powdered gluten could not be completely dispersed in the dilute acetic acid, and the particles, altho slightly swollen, sank rapidly to the bottom of the flask, leaving a fairly clear supernatant liquid. Nevertheless this supernatant liquid, which could be filtered water-clear through paper pulp, contained a large amount of dissolved or dispersed protein.

When a small amount of salt such as CaCl_2 or LiCl was added to this clear liquid, most of this protein settled out in fairly large aggregates and could be collected in a gummy mass on a stirring rod. The same result was obtained by neutralizing the acid extract with alkali. This material, altho coherent, was extremely soft, sticky, and inelastic. It was readily soluble in 50 to 70% alcohol or in strong acetic acid. This, of course, indicates that it consisted chiefly, if not entirely, of gliadin, the glutenin having remained in the undispersed residue. These experiments show that some alteration in the physico-chemical properties of glutenin has resulted from the drying procedure to which the crude gluten was subjected, and they suggest a simplified procedure for the preparation of gliadin in quantity. The next step, therefore, was to prepare a quantity of gliadin by the procedure just indicated, to re-dissolve and re-precipitate according to approved methods, and to ascertain the degree of purity of the product.

A large quantity of crude gluten was prepared in the customary manner from a baker's patent flour milled by a local commercial mill. Tap water was used in the kneading process. This gluten was thoroly dried in vacuo at 60 to 65°C., and ground as finely as possible in a coffee mill. A portion representing 400 gm.

of this material was treated with 16 liters of .07 N acetic acid for $2\frac{1}{2}$ hours, with vigorous shaking at frequent intervals. The suspension was then allowed to stand $1\frac{1}{2}$ hours, during which time the suspended material settled to the extent that about two-thirds of the supernatant liquid could be siphoned off. Approximately 10 more liters of .07 N acetic acid was then added to the residue, with stirring, and the insoluble portion again allowed to settle. This time, however, the suspended residue settled only about one-half instead of two-thirds of the distance to the bottom, and accordingly one-half of the liquid was siphoned off and added to the extract previously withdrawn. The total extract, nearly 15 liters in volume, was then filtered water-clear through a thick mat of filter paper pulp on a large Buchner funnel.

The clear liquid thus obtained was then treated with approximately 100 gm. of lithium chloride, this salt being used for reasons advanced by Dill and Alsberg (1925). There was an immediate precipitation of gliadin, which readily collected on the stirring rod in the usual manner. This was readily dissolved in 1 liter of ethyl alcohol of approximately 60% by volume, allowing for the water in the gliadin mass. The solution was quite free from cloudiness. After standing over night in an icebox, there was no deposit of "impure" gliadin, as in the method of Dill and Alsberg (1925), but merely a very slight deposit of sediment. The gliadin was then reprecipitated by pouring into absolute alcohol containing ether and a little lithium chloride. It was redissolved in 60% ethyl alcohol, reprecipitated in water containing lithium chloride, again redissolved in 60% alcohol and finally reprecipitated in absolute alcohol and ether. That the last two repurifications were necessary is doubtful. The gliadin was dried in vacuo at $50^{\circ}\text{C}.$, giving a snow white preparation which was readily reduced to a fine powder, and gave a permanently water-clear solution in dilute alcohol.

The yield in this case was 95 gm. This is, of course, far from a quantitative yield, but is satisfactory when the time and economy of the method are taken into account. Furthermore, evidence will be presented to show that the product is of an unusually high degree of purity.

The nitrogen content of this preparation, as determined by the Kjeldahl-Gunning method, was 17.54%, on the moisture-free basis. This is in exact agreement with Dill and Alsberg (1925), and endorses their opinion that Osborne's figure of 17.66 is slightly high. The percentage of ammonia nitrogen after acid hydrolysis

was 26.38, which agrees closely with values obtained by Blish (1916), Cross and Swain (1924), and Dill and Alsberg (1925). Further evidence of the high degree of purity of the product was the fact that on the hydrolysis of 1 gm. with acid, there was no blackening of the solution, beyond a light straw color, indicating a negligible amount of humin formation. Gortner and Blish (1915) found that the humin formed during the acid hydrolysis of proteins is chiefly a condensation product of tryptophane and carbohydrate. This gliadin preparation is therefore free from any carbohydrate material, as tryptophane is known to be a constituent of gliadin. The specific rotation in 70% alcohol was -92.0° , using a Schmidt and Haensch polarimeter, with white light filtered through at 1.5 cm. of a 6% potassium bichromate solution at 20°C . The rotatory dispersion at a wave length of 5461° Ang. units (mercury green line), at 20°C . was 113.5° . The ash content was .12%, which is exceedingly low.

The use of dilute acetic acid as a preliminary solvent for gliadin, under the conditions specified, appears to have an advantage over alcohol aside from the matter of economy already mentioned. It should make for a high degree of purity, especially from the standpoint of contamination by fats and lipid material. Such appears to have been the case with the preparation here reported, for an exceedingly pure product was obtained without extraction of the dry powdered material with alcohol and ether. This suggests an obvious procedure for the rapid purification of impure gliadin which has been prepared by the alcohol extraction method without extreme precautions such as were resorted to by Dill and Alsberg (1925).

Altho gliadin is readily soluble in dilute acetic acid, the use of this reagent for the preparation of pure gliadin from wheat flour appears at present to be confined to the procedure specified in this report. Altho its possibilities in other connections have yet to be thoroly investigated, certain observations are of interest.

If **untreated** flour is extracted with dilute acetic acid (.01 to 0.1N), about three fourths of the total protein is dispersed. This is considerably more than is extracted by **cold** 70% alcohol under the same conditions, and almost identical with the amount of protein extracted by **hot** 70% alcohol. From this acetic acid extract, gliadin is **not** readily recovered by adding salts, or by neutralizing the acetic acid with alkali, as is the case with similar extracts of dried crude gluten. Instead, the addition of salt causes an extreme milkiness, indicating that the gliadin comes out in exceedingly

fine aggregates. However, these will not settle out of suspension, nor can they be made to coalesce and gather together in a mass on a stirring rod, as is readily accomplished when dilute acetic acid extracts of dry crude gluten are similarly treated.

Why does gliadin extracted from flour by dilute acetic acid have physical properties which are thus different from gliadin similarly extracted from dry crude gluten? The only explanation which the writers can advance at this time is the possible protective action of the highly colloidal, non-nitrogenous gum which Gortner and Hoffman² have recently found in flour, and which they find can be removed by extraction with dilute potassium sulfate. That this is the explanation is further indicated by the fact that gliadin may be satisfactorily precipitated and collected on the stirring rod when salt is added to a dilute acetic acid extract of flour, **provided that the flour has first been extracted with dilute 5% potassium sulfate, and the potassium sulfate is then removed by several treatments with distilled water.** If this explanation is correct, it must, of course, be true that the gum is removed by the usual process of gluten-washing.

There remains to be reported that in the preparation of gliadin with the extraction of dry powdered crude gluten with dilute acetic acid as the preliminary step, certain precautions must be taken in the drying of the gluten, especially as regards temperature. When dried in the oven for 3 days at 80°C., the gliadin apparently became denatured or otherwise altered so that, altho it could be extracted with dilute acetic acid, and separated from starch and glutenin by filtration, it had lost its characteristic coherence and could not be gathered in a mass on the stirring rod. It could, however, be precipitated in fairly large aggregates by near-saturation with sodium chloride. Best results, with maximum yields, are obtained by drying at not to exceed 70°C., in a high vacuum. **Wet** crude gluten could not be made to serve as a starting point for the preparation of gliadin by this procedure, for in this case the gliadin and glutenin are **both** dispersed by the dilute acetic acid, giving an opaque suspension. Altho the glutenin does not appear to be very highly dispersed under these conditions, it cannot be satisfactorily removed by filtration.

² Private communication from Dr. R. A. Gortner.

Summary

When crude gluten, which has been dried in vacuo at 65 to 70°C., is ground to a powder and treated with .01 N to 0.1 N acetic acid, only gliadin is dispersed. The extract may be filtered water-clear, and the gliadin recovered by the addition of salt, or by neutralization with alkali. This furnishes a basis for preparing gliadin in quantity and of a high degree of purity. It is superior to the established method from the standpoint of economy (in time, expense, and alcohol) and simplicity of operations.

Literature Cited

- Bailey, C. H.
1925. The chemistry of wheat flour. p. 284. The Chemical Catalog Co., New York.
- Blish, M. J.
1916. On the chemical constitution of the proteins of wheat flour and its relation to baking strength. *J. Ind. and Eng. Chem.*, Vol. 8, pp. 138-144.
- Cross, R. J., and Swain, R. E.
1924. The amino acid distribution in proteins of wheat flours. *J. Ind. and Eng. Chem.*, Vol. 16, pp. 49-52.
- Dill, D. B., and Alsberg, C. L.
1925. Preparation, solubility, and specific rotation of wheat gliadin. *J. Biol. Chem.* Vol. 65, pp. 279-304.
- Gortner, R. A., and Blish, M. J.
1915. On the origin of the humin formed by the acid hydrolysis of proteins. *J. Am. Chem. Soc.* Vol. 37, pp. 1630-1636.
- Osborne, T. B., and Harris, I. F.
1906. The chemistry of the protein bodies of the wheat kernel. Part II. Preparation of the proteins in quantity for hydrolysis. *Am. J. Physiol.*, Vol. 17, pp. 223-230.
- Sharp, P. F., and Gortner, R. A.
1922. Physico-chemical studies of strong and weak flours. II. The imbibitional properties of the glutens from strong and weak flours. *J. Phys. Chem.*, Vol. 26, pp. 101-136.
1923. Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. *Minn. Agr. Exp. Sta. Tech. Bull.* 19.
- Upson, F. W., and Calvin, J. W.
1915. On the colloidal swelling of wheat gluten. *J. Am. Chem. Soc.*, Vol. 37, pp. 1295-1304.
- Wood, T. B.
1907. The chemistry of the strength of wheat flour. *J. Agr. Sci.* Vol. 2, pp. 267-277.

RELATION OF PROTEIN CONTENT TO BAKING QUALITY OF FLOUR FROM HARD RED SPRING AND DURUM WHEATS

By C. E. MANGELS

Department of Milling, North Dakota Agricultural Experiment
Station, Fargo, No. Dak.

(Received for publication March 5, 1926)

The superior bread-making properties of wheat flour as compared with flours from other cereals are evidently due to the peculiar properties of the protein or gluten of the wheat. While the important rôle of the gluten or protein constituent in determining the baking properties of wheat flour is generally recognized, there is considerable difference of opinion regarding the relation of the quantity of protein in flour and the baking quality. The protein content of wheat not only varies in quantity, but may also vary in quality. The baking properties of a flour may also be influenced by factors other than the protein content, and the relationship existing between protein content of flour and baking strength may therefore be obscured in some cases by variations in other factors.

The relationship between protein content and baking strength is of considerable commercial importance. This paper presents results of studies on data collected at the North Dakota Experiment Station over a period of years.

Literature

The protein or gluten content of "strong" or hard wheat flour is relatively higher than that of "weak" or soft wheat flours. Bakers prefer strong flours, because they will consistently produce good bread.

Stockham (1920) added dried gluten to the same flour in different amounts and obtained an increase in loaf volume due to the added gluten. He also found, however, that flour milled from wheat containing 15% or more protein was of poor baking quality and this inferior quality is attributed to the poor quality of the gluten in such wheats.

Thomas (1917) finds that flour shows a general increase in strength with increase in protein content, the only exception being with hard red spring wheat having a crude protein content of over 15 per cent.

Mangels and Sanderson (1925) found a positive correlation between protein content and baking quality (as measured by loaf volume) for the hard red spring wheat crops of 1921, 1922, and 1923.

Zinn (1923) made rather extensive correlation studies on American wheat varieties, using published data of the various experiment stations. He finds correlations for protein content and loaf volume of flour as follows:

Commercial varieties	No. of samples	Coefficient of correlation	
North Dakota spring wheats	119	-.0987	$\pm .0612$
Minnesota spring wheats	203	.2586	$\pm .0442$
Montana spring wheats	34	.3448	$\pm .1019$
Colorado spring wheats	48	.6130	$\pm .0603$
Montana winter wheats	91	.3620	$\pm .0614$
Ohio winter wheats	99	.4709	$\pm .0528$
Minnesota winter wheats	43	.6496	$\pm .0594$
Kansas winter wheats	43	.7956	$\pm .0377$
Pure strains			
North Dakota spring wheats	28	.3018	$\pm .1158$
Minnesota spring wheats	48	.5469	$\pm .0684$
Ontario spring wheats	16	.5752	$\pm .1129$
Wisconsin winter wheats	25	.3990	$\pm .1134$
Ohio winter wheats	25	.5560	$\pm .0932$

The only negative correlation shown is for North Dakota commercial varieties. Zinn used data from Bulletins 89 and 93, North Dakota Experiment Station, which reported results on crops of 1908, 1909, and 1910, and has evidently included the durum wheat samples. This would account for the negative correlation obtained.

The writer, using the data from Bulletins 89 and 93, obtained a correlation of $.523 \pm .062$ for 63 samples of North Dakota hard red spring wheat from the crops of 1908, 1909, and 1910, but when the durum samples from the same years were included, the same negative correlation reported by Zinn ($-.0987 \pm .0612$) was found.

Blish and Sandstedt (1925) found a significant positive correlation between protein content and loaf volume for Nebraska hard winter wheats.

Bailey (1924) found a correlation between protein content and baking strength of flour, but stated also "that it is evident that the loaf volume does not increase regularly with increasing protein content."

Correlation of Protein Content and Baking Strength of Flour

For correlation studies, the loaf volume is generally used as an index of baking strength, and is the best now available. For the present study the accumulated data on eleven crops of North Dakota wheat have been used. The studies reported are based on protein content and baking results for straight grade flours prepared on a small experimental mill.

For the baking tests, the same formula has been used throughout; and with the exception of the 1924 crop, practically the same baking procedure was used. In 1924, a straight dough procedure was substituted for the sponge method previously in use.

Protein data on flour for crops of 1919 and 1920 were not available. Hard red spring wheats and durum wheats have been studied separately, and the data for each crop year have been handled as a unit.

Correlation Between Protein Content and Baking Strength as Measured by Loaf Volume, of Hard Red Spring Wheat Flour

Table I gives the coefficient of correlation between protein content and loaf volume, for eleven crops of North Dakota hard red spring wheat. All coefficients of correlation are positive with the exception of crops of 1912 and 1913, but the negative coefficients obtained in 1912 and 1913 are in each case less than the respective probable error. The highest positive correlation coefficients are those for the crops of 1918 and 1924 and are respectively $.547 \pm .032$ and $.508 \pm .038$. The coefficient of $.473 \pm .036$, obtained for the 1916 crop, is next in order of magnitude, and is followed by coefficients of $.427 \pm .047$ and $.384 \pm .044$ for the crops of 1922 and 1915, respectively. The higher coefficients of correlation, with the exception of that for 1916, are found in years when the mean temperature of the growing season was favorable to good yields of grain, and in all five cases the rainfall during the growing season was adequate.

The crops of 1912 and 1913 show negative coefficients of correlation and the coefficient for 1917 is only $.187 \pm .046$, which is decidedly lower than coefficients obtained for the remaining eight crop years. It is significant that the seasons of 1917 and 1913 were dry. A majority of the samples of the 1912 crop were obtained from the rotation and fertility plots at Fargo, and while

the average rainfall for North Dakota was above normal in 1912, the rainfall at Fargo during the growing season was deficient.

Table I shows that the coefficient of correlation between protein content and loaf volume for six of the eleven crops studied (1915, 1916, 1918, 1922, 1923, and 1924) is positive and more than six times the respective probable error. The coefficients of correlation for two crops (1918 and 1924) are greater than .500, and for eight of the eleven crops studied, are greater than .300.

TABLE I
CORRELATION BETWEEN PROTEIN CONTENT AND LOAF VOLUME
OF HARD RED SPRING WHEAT FLOUR
Data on straight grade flour

Year	No. of samples	Average protein content	Average loaf volume	Coefficient of correlation	Probable error
		Per cent	cc.		
1912	72	12.97	2239	-.064	±.079
1913	186	11.52	2457	-.014	±.043
1914	123	11.61	2528	+.312	±.055
1915	172	10.10	2327	+.384	±.044
1916	212	12.44	2359	+.473	±.036
1917	197	13.17	2136	+.187	±.046
1918	217	13.17	2197	+.547	±.032
1921	128	15.02	2370	+.307	±.057
1922	136	11.93	2321	+.427	±.047
1923	194	13.10	2369	+.345	±.043
1924	170	11.79	2137	+.508	±.033

The data in Table I indicate a significant positive correlation between the protein content of flour and loaf volume or baking strength. The magnitude of the coefficients of correlation, however, indicates that quantity of protein in flour is not the sole factor which determines the baking strength of the flour. The quality of gluten in flour is known to be subject to variation, but quantity and quality of gluten are not the only factors which determine the baking strength of a flour. The principal function of gluten in a bread dough is to hold the gas produced by the yeast, but adequate gas production in a dough is also essential. The amount of sugar used in the usual baking formulas is an inadequate supply for the yeast, and sugar must be produced from the starch of the flour, during the fermentation process. The diastatic properties of flour, or the ability to produce sugar from the starch in the flour, may often be a limiting factor which will affect the baking strength.

Diastatic Properties of Wheat Flour

Rumsey (1922) studied the relation of diastatic activity to baking strength of flour. He found considerable variation and, in general, flours which gave superior loaves showed a relatively high diastatic value. Bailey and Sherwood (quoted by Bailey, 1925, p. 239) were able to improve the baking quality of flour from hard winter wheat by increasing the diastatic activity of the flour.

Table II shows variation due to location and variety in diastatic activity of straight grade flours milled from wheat of the 1923 crop. The diastatic activity was determined by the method devised by Rumsey. Kubanka durum, in all cases, shows the highest relative diastatic activity and Marquis the lowest. All three varieties show variation in diastatic activity due to location, and if Dickinson and Williston are compared, there appears to be a relation between rainfall and diastatic activity. The Fargo samples were produced on heavy clay soil, and this may account for the low diastatic activity of the Fargo samples.

TABLE II
VARIATION IN DIASTATIC ACTIVITY OF WHEAT OF THE 1923 CROP
Data on straight grade flour.

Location	Maltose produced			Total precipitation 1923	Rainfall June and July 1923
	Marquis	Kota	Kubanka		
	Mg.	Mg.	Mg.	In.	In.
Fargo	57.4	103.5	160.1	19.05	9.07
Dickinson	90.1	164.5	240.6	19.67	9.16
Williston	75.0	114.3	221.4	17.00	7.31

The relation to rainfall or supply of moisture is indicated by data secured at Williston in 1923 on irrigated and non-irrigated plots. (See Table III.) Flour milled from wheat produced on irrigated plots shows a relatively higher diastatic activity than flour from wheat produced without irrigation. As the soil and general climatic conditions are the same, and water supply the only variable, the data indicate a relation between water supply and diastatic activity.

TABLE III
EFFECT OF IRRIGATION ON DIASTATIC ACTIVITY OF WHEAT FLOUR
Data on straight grade flour from wheat grown at Williston, No. Dak., in 1923.

Variety	Maltose produced	
	Non-irrigated plots	Irrigated plots
	Mg.	Mg.
Marquis	75.0	83.4
Kota	114.3	217.8

Poor baking quality in flour may be due to a relatively low diastatic activity. The data in Tables II and III indicate that the diastatic activity of wheat flour is influenced by soil and climatic conditions—particularly the moisture supply. As low correlation coefficients between protein content and loaf volume of flour are found in the relatively dry years of 1917 and 1913, it is probable that in these crops the diastatic activity was of relatively greater importance as a factor in determining baking strength on loaf volume, than in other years.

It is now generally recognized that baking strength of flour is not controlled by a single factor. The protein content of flour is an important factor in determining baking strength, but at present we can recognize at least two other factors—quality of gluten and diastatic activity. When hard red spring wheat is produced under normal or favorable climatic conditions, the average gluten quality and diastatic activity are satisfactory, but under unusual or unfavorable climatic conditions, gluten quality and diastatic activity may be so reduced that they assume relatively greater importance as quality factors, and the relation existing between protein content and baking quality may be obscured.

Durum Wheat Flours

The baking results obtained with durum wheat flours are interesting, as durum wheats on the average have a protein content equal to that of the hard red spring wheats, or slightly higher. If the quantity of protein present were the sole factor affecting the baking strength of flour, we would expect durum wheat flours to be very high in baking quality; however, they show relatively poor baking quality. Figure 1 shows that over a period of years the average loaf volume of durum wheat flours has been consistently much lower than that of hard red spring wheat flours for samples milled and baked at the North Dakota Experiment Station.

Protein Content and Loaf Volume of Durum Wheat Flours

Table IV gives the coefficients of correlation found between protein content and loaf volume for ten crops of North Dakota durum wheat. The correlation is positive for nine of the ten crops studied, but the coefficients of correlation show a wide variation—from $+0.806$ to -0.559 . For six crops (1914, 1915, 1917, 1918, 1921, and 1924) a positive correlation greater than $.300$ was obtained, but owing to the small number of samples used, no positive coefficient is more than six times the probable error.

The degree of correlation between protein content and loaf volume found in durum wheat flours is somewhat less than that obtained for hard red spring wheat flours. The magnitude of the

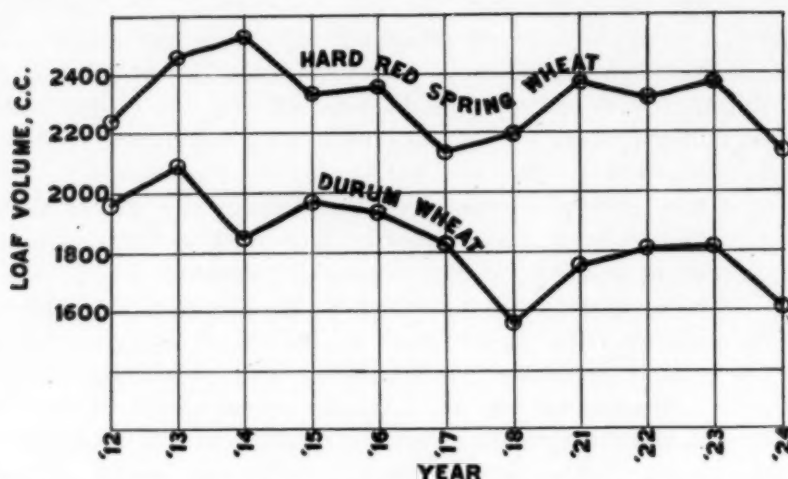


Fig. 1. Comparison of Average Loaf Volume of Hard Red Spring and Durum Wheats

correlation coefficient, as in case of hard red spring wheat flours, indicates that protein content is not the sole factor in determining loaf volume or baking strength.

TABLE IV
CORRELATION BETWEEN PROTEIN CONTENT AND LOAF VOLUME
OF DURUM WHEAT FLOUR
Data on straight grade flour

Year	No. of samples	Average protein content	Average loaf volume	Coefficient of correlation	Probable error
		Per cent	cc.		
1913	43	12.00	2088	-.559	± 0.070
1914	35	11.46	1850	+.473	± 0.088
1915	32	10.38	1975	+.519	± 0.087
1916	52	12.38	1934	+.101	± 0.092
1917	19	13.74	1827	+.470	± 0.120
1918	61	13.71	1564	+.380	± 0.073
1921	79	14.23	1756	+.305	± 0.068
1922	72	12.25	1814	+.206	± 0.076
1923	59	14.04	1820	+.229	± 0.083
1924	32	12.70	1615	+.806	± 0.059

The poor baking quality of durum wheat flours cannot be ascribed to low protein content, as the durum wheats on the average equal or slightly exceed the hard red spring wheats in protein content. The diastatic activity of durum wheat is also relatively high. Table III shows that flour from Kubanka durum exceeds that from Marquis and Kota in diastatic activity.

The relatively low baking strength of durum wheat flour is evidently due to the inability of the gluten to hold the gas produced, and in this case poor baking quality is evidently due to a poor quality of gluten.

Summary

A significant positive correlation was found between protein content and baking strength of hard red spring wheat flour (as measured by loaf volume), for eight of eleven crop years.

The magnitude of the coefficient of correlation indicates that protein content is not the sole factor which determines baking strength of hard red spring wheat flours; the quality of the gluten and the diastatic activity of the flour are also important factors.

Durum wheat flours consistently average lower in loaf volume than hard red spring wheat flours.

A positive correlation greater than .300 was found between protein content and baking strength of durum wheat flours (as measured by loaf volume) for six of ten crop years.

The relatively low baking quality of durum wheats is evidently due to poor quality of gluten.

The writer is indebted to Dr. C. H. Bailey, of Minnesota, for suggestions regarding influence of variation in diastatic activity of flour.

Literature Cited

- Bailey, C. H.
1924. Report of operation. State Testing Mill, crop season of 1922. Minn. State Dep. Agr. Bull. 34.
1925. The chemistry of wheat flour. Chemical Catalog Co., New York, (note p. 239).
- Blish, M. J., and Sandstedt, R. M.
1925. Viscosity studies with Nebraska wheat flours. Cereal Chem. Vol. II, pp. 199-202.
- Mangels, C. E. and Sanderson, T.
1925. The correlation of the protein content of hard red spring wheat with physical characteristics and baking quality. Cereal Chem. Vol. II, pp. 107-112.
- Rumsey, L. H.
1922. Diastatic enzymes of wheat flour and their relation to flour strength. Am. Inst. Baking. Bull. 8.
- Stockham, W. L.
1920. Some factors related to the quality of wheat and the strength of flour. No. Dak. Agr. Exp. Sta. Bull. 139.
- Thomas, L. M.
1917. A comparison of several classes of American wheats and a consideration of the factors influencing quality. U. S. Dep. Agr. Bull. 557.
- Zinn, J.
1923. Correlations between various characters of wheat and flour as determined from published data from chemical, milling, and baking tests of a number of American wheats. J. Agr. Res. Vol. 23, pp. 529-548.

A RAPID ELECTROMETRIC METHOD FOR THE MEASUREMENT OF HYDRION CONCENTRATION OF FLOUR-WATER SUSPENSIONS

H. J. DENHAM, M.A., D.Sc. (Oxon.)

and

G. W. SCOTT BLAIR, B.A. (Oxon.) A. I. C.

Research Department, Messrs. Henry Simon Ltd., Manchester,
England

(Received for publication March 8, 1926)

The following is a method of estimating the hydrion concentration of flour suspensions which has been in use in these laboratories for twelve months, and which appears to have considerable advantages over the hydrogen electrode as regards simplicity and rapidity in attaining equilibrium, even as compared with the Bailey electrode (1920), which is largely used by other cereal chemists in this country. The method is an adaptation of the quinhydrone electrode first described by Biilmann, and which is rapidly coming into favor in many fields of biochemical and other work.

It is not claimed that a high degree of accuracy is obtained, as it is considered that (owing to errors of sampling and heterogeneity of material) readings of less than one millivolt are not reliable.

The quinhydrone electrode.—Figure I shows a simple electrode vessel with one side syphon tube, fitted at the neck with a rubber bung through which passes a glass tube into the lower end of which is sealed a piece of platinum of any convenient thickness and diameter, e.g., 2 cm. x 1 cm. wide, and preferably of fairly stout gauge and rigidly supported. This platinum should be left bright and not coated with platinum black. The free end of the syphon dips into a small connecting vessel containing saturated potassium chloride, and into which also dips another electrode vessel (which, for convenience, is of exactly the same shape and size) containing a saturated calomel electrode of the recognized type. As a matter of convenience the whole is immersed in a water thermostat at $25 \pm .02^\circ\text{C}$. This accuracy of temperature control is unnecessary, but eliminates the temperature variable in calculating results. Potential differences between the electrodes are measured on a Cambridge Instrument Company's potentio-

meter of standard construction which can be read to the nearest 0.5 millivolt.

Fig. 1.

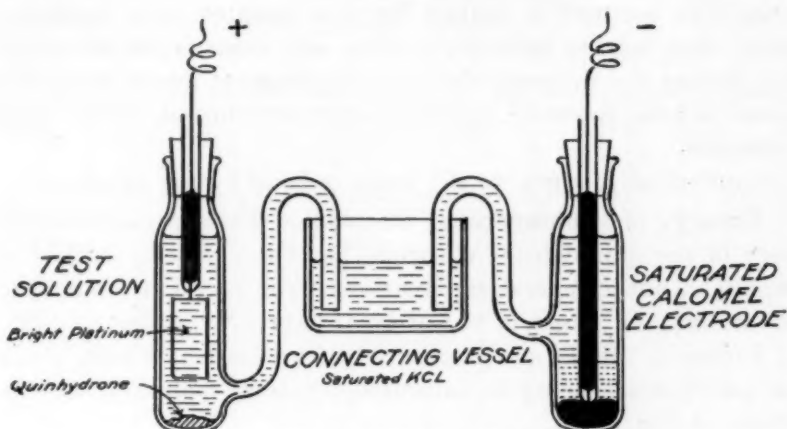


Fig. 1 Quinhydrone Electrode

Method of use.—A convenient amount of the liquid to be examined is placed in the quinhydrone electrode vessel and a few crystals of pure quinhydrone are shaken up with it. The platinum electrode, having been washed in 'conductivity water' is flamed over an alcohol flame and inserted. By careful manipulation of the rubber bung the liquid is forced to the tip of the syphon tube, which is then immersed in the saturated KCl of the connecting vessel. As soon as the temperature of the thermostat has been attained, readings may be taken on the potentiometer. If a thermostat is not used, readings may be taken at once, but a slight correction must be made for temperature.

Standardization.—The electrode was carefully calibrated for a number of known pH values which were obtained by use of Clark and Lubs' potassium phthalate-sodium hydroxide buffer solutions, and the results plotted as a curve of pH against voltage, at a constant temperature of 25°C. The resultant curve is linear, and has been repeated on at least four different occasions.

Measurement of flour suspensions.—It is possible to take measurements directly on flour and water mixtures of convenient dilution, but it has been found that there is a tendency for these mixtures to show a slight but continuous potential drift, due to leaching out of substances affecting the pH of the whole. For this reason it is preferred to use the standard extraction method as used in these laboratories for several other purposes. In this, 20 grams of flour are mixed intimately with 200 cc. 'conductivity water'

made in a Hartley-Bourdillon type of still, the mixing being done in a flask of borosilicate glass, fitted with a clean bung of well-aged rubber kept exclusively for the purpose and tested for buffer action. The mixture is shaken for five minutes in a mechanical shaker, then poured into 10-cc. tubes and centrifuged electrically for a further five minutes, the clear supernatant liquor then being poured into the electrode vessel and the procedure described above is followed.

Conversion of e.m.f. to pH units is done by the graph.

Theory of quinhydrone electrode.—The physico-chemical theory of the quinhydrone electrode has not yet been worked out completely, but a general explanation of the function of the quinhydrone may be found in the original papers by Biilmann (1921) and Sørensen, (1921) and is concisely expressed by Clark, (1922). The quinhydrone being in saturated solution, maintains an equilibrium of the type:

Quinhydrone \rightleftharpoons quinone + hydroquinone,
the position of which is independent of the absolute amount of quinhydrone present.

The pH is related to the e.m.f. against calomel by a relation of the type:

$$\text{pH} = \frac{\text{Constant} - \text{e.m.f. observed}}{.1985T} \dots\dots\dots (1)$$

where T is the absolute temperature.

The constant, obtained by means of the buffer curve already described, has a value 454.5, so that at 25°C, the equation becomes

$$\text{pH} = \frac{454.5 - \text{e.m.f. observed}}{59.16} \dots\dots\dots (2)$$

Effect of temperature.—The following table obtained from equation (1) shows to what extent variations in temperature will affect the e.m.f.'s measured.

Values of pH corresponding to an e.m.f. of 100 mv. at various temperatures.

Temperature (°C)	pH
10	6.310
15	6.200
20	6.101
25	5.992

Range of Applicability.—The quinhydrone electrode has been found satisfactory over the whole range of pH's occurring in flour extracts and the "salt error" mentioned by Biilmann is not appreciable at the concentrations used.

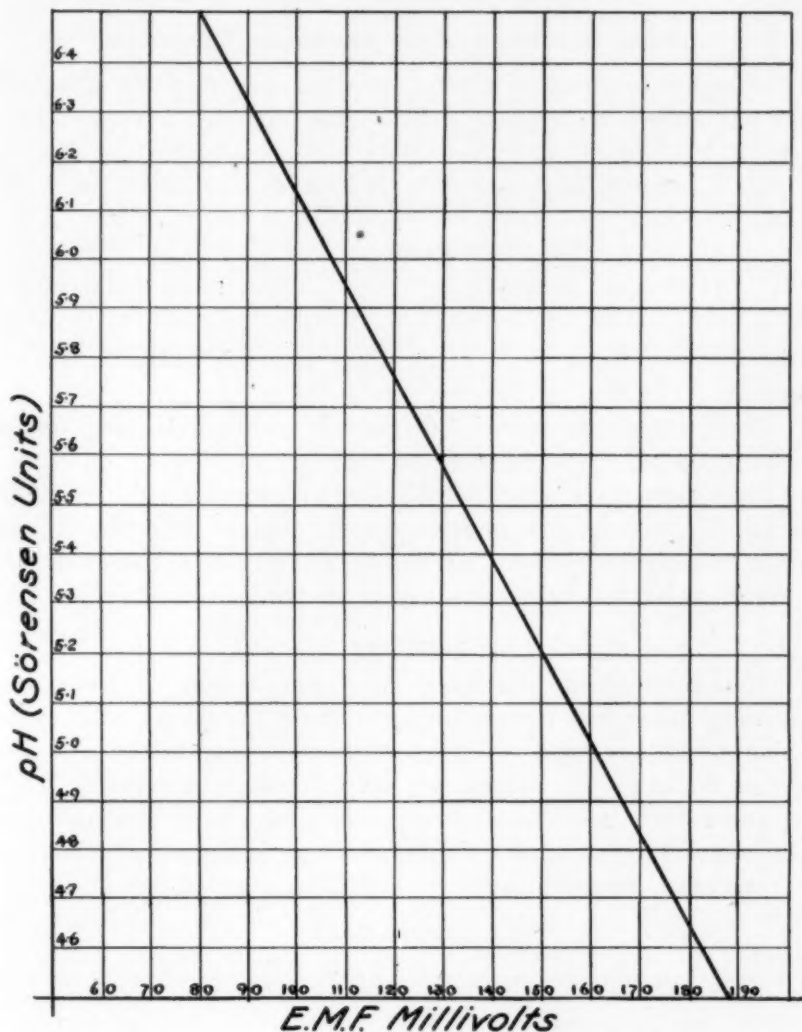


Fig. 2. pH Conversion Curve of Quinhydrone (Saturated Calomel Electrode) at 25°C.

The use of the quinhydro-quinhydrone electrode which Biilmann advocates to obviate the salt error is not, therefore, needed, but experiments on this electrode failed to give consistent results with flour extracts. (In the quinhydro-quinhydrone electrode, the

solution is saturated not only with quinhydrone, but also with quinone and hydroquinone.)

The other two electrodes depending on the same principle, namely, the hydroquinhydrone (hydroquinone and quinhydrone) and the quin-quinhydrone (quinone and quinhydrone) were also tried, but did not give consistent results with flour extracts.

Standardization Against Hydrogen Electrode

In order to show that the quinhydrone electrode gave the same pH values for a given flour as the standard hydrogen electrode, a Hildebrand hydrogen electrode was set up against the saturated calomel, and the pH's of two flour extracts were determined.

Results*

	Hydrogen electrode pH	Quinhydrone electrode pH
Flour 1	6.00	6.00
Flour 2	5.33	5.35

As the allowable error of 1 mv. on the quinhydrone corresponds to about .02 pH unit, these results are satisfactory.

The quinhydrone electrode has been frequently and repeatedly checked by colorimetric methods in the course of routine work, and is in entire agreement within such limits of accuracy as can be observed by this rather insensitive method.

Summary

The quinhydrone electrode does not appear to have been previously described for the determination of the pH's of cereal products. In this paper is given a short description of a simple form of the electrode suitable for work on flour extracts.

The results are shown to be consistent with the hydrogen electrode and, in view of its simplicity and robustness, it is to be recommended for such work.

Literature Cited

- Bailey, C. H.
1920. A simple hydrogen electrode. *J. Am. Chem. Soc.* Vol. 42, pp. 45-48.
- Biilmann, E.
1921. Sur l'hydrogénation des quinhydrone. *Ann. chim. ser. 9, t. 15, p. 109-157.*
- _____ and Lund, H.
1921. Sur l'électrode a quinhydrone. *Ann. chim. ser. 9, t. 16, p. 321-340.*
- Clark, W. M.
1922. The determination of hydrogen ions. 2nd ed. Baltimore (note p. 290).
- Sørensen, S. P. L., Sørensen, M., and Linderström-Lang, K.
1921. Sur l' "erreur de sel" inhérente à l'électrode de quinhydrone. *Ann. chim. ser. 9, t. 16, p. 283-320.*

CONTROL OF DIASTATIC ACTIVITY IN WHEAT FLOUR.

II. EXPERIMENTS WITH FLOUR MILLED ON A COMMERCIAL SCALE¹

By R. C. SHERWOOD AND C. H. BAILEY

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, St. Paul, Minn.

(Received for publication, February 15, 1926)

The earlier milling experiments were conducted on a small scale, using 2-kilogram portions of wheat which were ground with the laboratory roller mill. Experiments were then conducted with 3000-pound samples which were milled in the Minnesota State Experimental Flour Mill. This is a 150-barrel flour mill built with small sized commercial machinery, including special devices for determining the losses in cleaning wheat, and the exact yields of all products. Bailey (1922) has described the equipment of the mill and the methods employed for the milling tests of wheat.

Source and description of wheat.—The wheat chosen for the experiments on a commercial scale was of the Turkey Red variety of hard winter wheat grown in Montana. Examination of a sample of the wheat and of the flour milled from a 2000-gram sample on the small experimental mill, showed that it was high in protein and fairly low in diastatic activity, exactly the conditions desired for this study. The wheat graded No. 1 Hard Winter, dockage 1 per cent, protein 13.82 per cent, moisture 10.86 per cent.

Several bushels of the wheat were allowed to germinate for three days, using the apparatus and the method described in the first section. A photograph taken of representative kernels of the germinated wheat appears as Figure 3, and illustrates the length of plumule and rootlets. The wheat was then carefully dried, scoured to remove the sprouts and roots, and preserved for supplementing the diastatic power of the normal wheat.

Milling tests.—Milling tests were made with 3000-pound lots in each instance. Normal, or ungerminated, wheat was milled in order to obtain a control flour, and then a like quantity containing 2 per cent of germinated wheat was milled under the same conditions. It was found most convenient to add the germinated kernels to the normal wheat as the latter was discharged from an

¹ Published with the approval of the Director as Paper No. 602, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by R. C. Sherwood to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1925.

automatic, recording scale which dumps 30-pound charges. A predetermined weight of germinated wheat was added gradually each time the scale dumped, thus insuring that, with the subsequent movement of the grain on its way through the mill to the break rolls, it would be uniformly mixed.

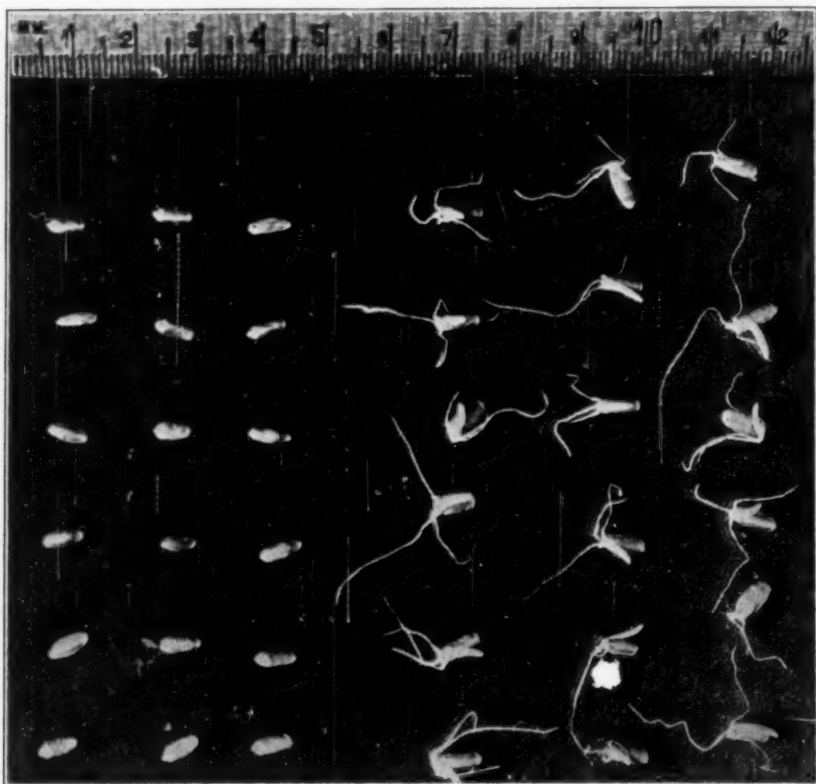


Fig. 3. Hard Winter Wheat Kernels, Ungerminated, and Germinated 3 Days

The wheat was washed, scoured, and tempered, then reweighed on a second automatic scale before milling, in order that the milling yields could be estimated accurately and calculated to the basis of the original moisture content of the wheat. Four products were manufactured, straight grade flour, low grade or red dog flour, bran, and shorts.

Determinations of diastatic activity were made on the flour manufactured in the two milling tests. The results showed that the activity had been increased from 126 in the control flour to

204 by the addition of 2 per cent of germinated wheat, and indicated that 3 per cent could be safely added. Consequently another lot was milled a few days later, in which 3 per cent of germinated wheat was incorporated, and the resulting flour exhibited a diastatic activity of 241 units.

The data collected during the milling tests were recorded and are reported in Table VIII in the form used by Bailey (1922).

TABLE VIII

RESULTS OF MILLING TESTS OF HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Germinated wheat	Per cent 0	Per cent 2	Per cent 3
Screenings from receiving separator	0.73	0.73	0.73
Screenings from milling separator	2.30	1.96	2.41
Total screenings corrected	3.02	2.68	3.12
Moisture before tempering	10.8	10.8	10.8
Moisture after tempering	15.0	16.2	15.8
Yield of products calculated to basis of original moisture content of wheat			
Straight flour	76.33	78.02	73.00
Total feeds	27.80	27.48	26.56
Total products	104.13	105.50	102.56

Yields of flour did not vary in order of percentage of germinated kernels. The wheat containing 2 per cent of sprouted kernels yielded more flour than the control, while the 3 per cent yielded nearly the same amount as the control. These data are not indicative of any substantial effect of such small quantities of germinated kernels upon milling yields. Previous tests with larger percentages of germinated wheat showed a reduced yield of flour, and the same results might be anticipated here; but if such is the case, the reduction is too small to be noticeable. No difficulty whatever was experienced in the milling or handling of any of the products. These facts are worthy of mention, since data which follow establish the fact that a decided improvement in the baking qualities of the flour was effected by adding a quantity of germinated wheat too small to be of significance in its effect upon the milling process. The three flours thus manufactured were then subjected to extensive examination in order to determine their relative value.

Determination of diastatic power.—Diastatic activity determinations, made as previously described and recorded in Table IX, showed a regular and substantial increase in the power of producing maltose, with the addition of germinated wheat. It will be noticed that there is a slight increase in the percentage of reducing

sugar in the flour, but that the quantity of reducing sugar produced, reported as diastatic activity in terms of Rumsey's units, was increased more than 60 per cent with the addition of 2 per cent, and nearly 100 per cent with the addition of 3 per cent of germinated wheat. This upper level of diastatic power is within the range which included the best baking flours of Rumsey's series.

TABLE IX

DIASTATIC ACTIVITY OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Germinated wheat	Original maltose per 10 gm. flour	Diastatic activity as maltose produced by 10 gm. flour
Per cent	Mgm.	Mgm.
0	76.0	126.4
2	79.2	203.6
3	86.0	241.2

Since the protein content of this wheat was entirely adequate for the production of good flour, the acceleration of the mechanism for producing available sugars for yeast fermentation should result in a better quality of flour, as determined by the tests which we apply, providing that there has not been impairment in some other particular. An increase in the gas-producing power of such flour when mixed into a dough may be anticipated but it is important to ascertain also the effects of the addition of sprouted grain upon the gas-retaining power of the gluten proteins. This phase of the problem was attacked from several angles, namely, determination of the gas-producing and gas-retaining power in practical tests with doughs prepared in the usual manner; estimation of the nitrogenous compounds of lower complexity than proteins, as an indication of protein hydrolysis, and estimation of the activity of proteases in digested samples of the flour by titration of amino compounds, using Sorenson's formol titration method; examination of the colloidal properties of the flour proteins, as evidenced by the effects of imbibition upon the viscosity of a flour-water suspension; and finally baking tests of the flours, both in the laboratory and in small commercial quantities.

Gas-producing and gas-retaining power.—Bailey and Johnson (1924b) have devised a method for measuring during fermentation the rate of the expansion of a dough, and also the rate at which carbon dioxide is lost from the dough. Since this method proved to be useful in determining the properties of fermenting dough, it was applied in the study of the flours made from wheat to which varying amounts of germinated wheat had been added.

TABLE X
INCREASE IN VOLUME OF DOUGH, VOLUME OF CARBON DIOXIDE LOST FROM DOUGH, AND SUM OF THESE VOLUMES AS DETERMINED AT 10
MINUTE INTERVALS FOR 250 MINUTES

Time Min.	Flour No. 894 Control, from ungerminated wheat				Flour No. 895 From mixture containing 2 per cent germinated wheat				Flour No. 912 From mixture containing 3 per cent germinated wheat			
	Increase in volume of dough plus CO ₂ lost from dough		CO ₂ lost from dough		Increase in volume of dough plus CO ₂ lost from dough		CO ₂ lost from the dough		Increase in volume of dough plus CO ₂ lost from dough		CO ₂ lost from the dough	
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
0	0	0	0	0	0	0	0	0	0	0	0	0
10	3	3	0	0	4	4	0	0	6	5	0	0
20	9	9	0	0	10	9	1	1	13	12	1	1
30	14	14	1	1	17	16	1	1	24	22	2	2
40	22	21	1	1	25	23	2	2	33	31	2	2
50	31	29	2	2	33	31	2	2	44	41	3	3
60	42	40	2	2	43	40	3	3	56	53	3	3
70	52	49	3	3	54	51	3	3	68	65	3	3
80	62	59	3	3	64	61	3	3	81	77	4	4
90	73	70	3	3	75	71	4	4	95	89	5	5
100	85	81	4	4	88	83	5	5	108	103	6	6
110	96	91	5	5	100	95	5	5	122	115	7	7
120	109	103	6	6	112	106	6	6	136	128	8	8
130	121	115	6	6	125	118	7	7	151	141	10	10
140	134	126	8	8	139	130	9	9	166	154	12	12
150	147	136	11	11	152	142	10	10	182	166	15	15
160	162	148	14	14	166	153	13	13	197	175	22	22
170	175	159	16	16	179	163	16	16	212	180	32	32
180	189	168	21	21	194	172	22	22	229	187	42	42
190	203	174	29	29	209	178	31	31	245	189	56	56
200	217	178	39	39	223	181	42	42	260	192	68	68
210	230	180	50	50	237	184	53	53	275	196	79	79
220	245	183	62	62	252	185	67	67	290	201	89	89
230	259	186	73	73	266	186	80	80	307	200	107	107
240	272	185	87	87	279	188	91	91	321	199	122	122
250	285	188	97	97	292	192	100	100	335	201	134	134

Doughs mixed according to the procedure of Bailey (1916) with 2 per cent of yeast and 3 per cent of sugar, were used for the determinations. The temperature of the water bath was maintained at 28°C. Readings were made every 10 minutes for a period

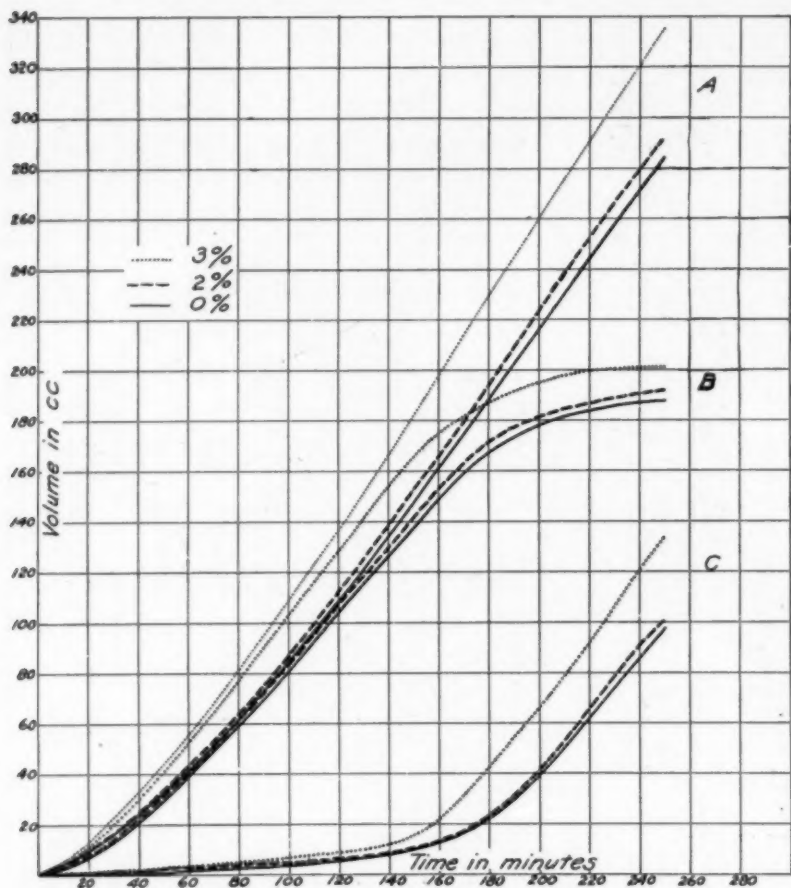


Fig. 4. Changes in volume which occur in systems containing fermenting doughs. Flours Nos. 894, 895, and 912 milled from 0, 2, and 3 per cent of germinated wheat mixtures respectively. Curves A represent the sum of the increase in volume of dough and volume of carbon dioxide lost from the dough; curves B the increase in volume of the dough; and curves C the volume of carbon dioxide lost from the dough

of 250 minutes. The control flour and the flours manufactured from 2 per cent and 3 per cent germinated wheat mixtures were thus compared. The results, which are averages of triplicate determinations, are given in Table X. Curves constructed from this table (Fig. 4) show the results to better advantage. During fermentation the three doughs showed a regular increase in volume

of dough plus gas liberated, the rate of increase being progressively higher with the flours of higher diastatic activity.

The dough from flour containing 3 per cent of germinated wheat began to lose carbon dioxid in quantity about 20 minutes sooner than that from the flour containing 2 per cent of germinated wheat, indicating that the time for the "first punch" would come sooner, and consequently the total time of fermentation would be shorter. The dough prepared with the flour containing 3 per cent of germinated wheat lost a greater quantity of gas in the same length of time, but as more gas was produced, the actual volume of the dough was 13 cc. greater at the expiration of 250 minutes. The dough prepared with the control flour, containing no germinated wheat, began to approach its maximum volume after about 200 minutes, while that with the 3 per cent admixture reached the same volume about 35 minutes sooner.

It is concluded from these gas-production and gas-retention experiments that the addition of 2 and 3 per cent of germinated wheat to the wheat mixture increased the gas-producing capacity of the flour and the rate of gas production so that the fermentation time may be shortened and in no way impaired the gas-retaining power, but rather improved it, as a larger volume of dough was obtained.

TABLE XI

TOTAL NITROGEN AND NITROGEN IN FRACTIONS NOT PRECIPITATED BY SN AND CU REAGENTS IN FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germin- ated wheat	Nitrogen			
		Total	Water- soluble	Sn-non- precip- itable	Cu-non- precip- itable
	Per cent	Per cent	Per cent	Per cent	Per cent
894	0	2.13	0.236	0.188	0.027
895	2	2.13	0.235	0.193	0.027
912	3	2.10	0.232	0.190	0.031

Determination of nitrogen in fractions not precipitated by various reagents.—In the preliminary trials in which large percentages of germinated wheat were used, it was found that there were appreciable increases in soluble nitrogen, peptid, and amino nitrogen, as determined by fractional precipitation. The larger quantities of simple nitrogen compounds were attributed to protein hydrolysis by proteases added with the germinated wheat. These results indicated, however, that less than 5 per cent of wheat germinated for 3 days would not cause a substantial change in nitrogen distribution. Nitrogen determinations were made, therefore, using the same procedure as outlined previously. The results of the tests are shown in Table XI, where the percentage of each

form of nitrogen in the flour is given. A survey of these data indicated a very slight increase in nitrogen fractions not precipitated by Sn and Cu reagents as the percentage of germinated wheat increased, but the difference was not great enough to be of significance.

Protease activity determinations.—The activity of proteases in the flour was studied, using Sorenson's (1907) formol titration method for determining the extent of hydrolysis during a long digestion of a flour-water suspension. The acidity due to carboxyl groups in compounds existing in the flour was first determined. A 25-gram sample of flour was suspended in 100 cc. of distilled water saturated with toluene, and allowed to stand with occasional shaking for 90 minutes. The suspension was centrifuged, filtered, and 50 cc. of the clear liquid titrated with 0.1 N sodium hydroxide using phenolphthalein as indicator. Then 10 cc. of neutralized 40 per cent formaldehyde was added, and a second titration made with 0.1 N sodium hydroxide. The difference between the two titrations indicated the quantity of alkali required to neutralize the free carboxyl groups. Two samples of each flour were then suspended in water in the same manner and digested, the first for 46 hours at 35°C. and the second for 6 days at 35°C. Determinations of acidity were made by the method just described.² These results are recorded in Table XII.

TABLE XII
PROTEASE ACTIVITY OF HARD WINTER WHEAT FLOURS MILLED FROM WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT, AS DETERMINED BY FORMOL TITRATION AFTER AUTOLYTIC DIGESTION OF 1:4 FLOUR-WATER SUSPENSIONS

Flour Lab. No.	Ger- minated wheat	0.1 N sodium hydroxide required to neutralize extract of 25 grams flour		
		Undigested	Digested 46 hours	Digested 6 days
	Per cent	cc.	cc.	cc.
894	0	1.3	2.0	3.6
895	2	1.3	2.1	3.8
912	3	1.5	2.2	3.9

Inspection of these data shows that there is a definite increase in the amount of sodium hydroxide required to neutralize the extract after 46 hours digestion and, as would be expected, a greater increase after 6 days digestion. This shows that proteases are present in the flours. Comparing the three flours, however, it is seen that flours 895 and 912 required no substantial increase of alkali for neutralization in either of the series of digested samples.

The results indicate only a slight increase in protease activity in the flours milled from wheat mixtures containing 3 per cent or less of germinated wheat after a long digestion. It should be

² Acknowledgment is made of the kind assistance of Mr. Andrew Cairns.

mentioned that in bakery practice the doughs never stand this length of time; 5 hours or less is the usual fermentation time of bread doughs and, during this comparatively short interval, only small changes in the amount of titrable nitrogen would be anticipated.

Activity of enzymes is influenced by the ratio of flour to water as well as by the time of digestion. It is certain that the 1:4 suspension used for the tests of protease action will permit more rapid hydrolysis than the 1:0.6 dough prepared for bread purposes. The conclusion is drawn from this study of protease activity that the amount of proteases contributed by the germinated wheat used in preparing these flours is insignificant in its effect upon bread doughs.

Viscosity determinations.—A further indication of the effects of the addition of germinated wheat upon protein quality may possibly be indicated by viscosity determinations, using the method of Gortner (1924). This method involves washing a definite weight of flour, first with 1000 cc. of distilled water with agitation at 15-minute intervals for 1 hour, decantation of the supernatant liquid when the insoluble residue has settled, and then a second washing with 500 cc. of distilled water, after which the residue from decantation is diluted to exactly 100 cc. A measured portion of this thoroly mixed suspension is transferred to the cup of the viscometer, and the reading taken.

Concentrations of 12, 15, 18, and 21 grams were examined in this manner, and from the logarithms of the results the constants *b* were determined. Table XIII contains both the actual reading in degrees MacMichael and the constants *b*.

TABLE XIII

VISCOSITY AS DEGREES MACMICHAEL AND CONSTANT *b* OF FLOURS MILLED FROM HARD WINTER WHEAT WITH ADMIXTURE OF GERMINATED WHEAT
Observations Made Upon Washed Flour Suspensions of Varying Concentrations

Flour Lab. No.	Ger- minated wheat Per cent	Viscosity				Viscosity constant <i>b</i>
		Flour concentration				
		gm.				
		12	15	18	21	
		M°	M°	M°	M°	
894	0	27	50	84	124	2.73
895	2	28	55	90	142	2.88
912	3	22	42	67	115	2.87

The flours milled from the wheat containing germinated wheat gave slightly higher results, expressed as constant *b*, but the differences are so slight as to indicate no appreciable change in such gluten properties as are measured by this method of examination.

Baking tests in the laboratory.—The flours were subjected to baking tests in the laboratory, following the method described by Bailey (1922) and using the following formula:

	Grams	Per cent
Flour	350	100
Yeast	10.5	3
Sugar	10.5	3
Salt	5.25	1.5
Fat	5.25	1.5
Water	sufficient	

In Table XIV the results of the test are listed.

TABLE XIV
LABORATORY BAKING TESTS OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germin- ated wheat	Fermentation period	Loaf volume	Color	Texture
	Per cent	Min.	cc.	Score	Score
894	0	340	2000	97	98
895	2	332	2100	98	99
912	3	323	2150	99	100

The fermentation time was shortened somewhat with the germinated wheat samples, and it was in the proofing period that most of the time was saved, as the diastatic enzymes were accelerated at the higher temperature of the proofing thermostat with more rapid production of carbon dioxide. The loaf volume was progressively larger with an increase in the proportion of germinated wheat from 2 to 3 per cent. An improvement was noted in color and texture of the crumb, the difference in color being attributed to the larger volume and improved texture, which result in greater dispersion of colloidal particles and consequent lighter color. The better texture is evidence that the quality of the gluten was at least as good, if not slightly better in the germinated wheat flour. Somewhat browner crusts were obtained with Nos. 895 and 912 because of the larger amount of sugars present when the loaves were ready for the oven. Photographs of cross-sections of the loaves are shown in Figure 5.

Reducing sugar in fermenting doughs.—In order to determine whether the addition of germinated wheat was responsible for production of reducing sugars in greater quantity during fermentation of a dough, flours 894, and 912 were doughed in the usual manner with 3 per cent yeast and 3 per cent sugar. The total reducing sugar calculated as maltose was determined immediately after mixing, at the end of two hours, and after five hours. These results are given in Table XV.

TABLE XV
REDUCING SUGAR AS MALTOSE IN FERMENTING DOUGHS

Flour Lab. No.	Germin- ated wheat	Maltose at intervals during fermentation		
		When mixed	2 hours	5 hours
894	0	338	430	292
912	3	402	484	380

The 3 per cent flour contained 64 milligrams more maltose than the control at the outset, and 88 milligrams more at the end of 5 hours. This justified the conclusion that there was more fermentable sugar available for the yeast in the dough made from germinated wheat flour throughout the fermentation period. Likewise, at the end of the fermentation period there was a larger quantity present, which indirectly contributed to greater expansion in the oven and browner crust. Reference to Figure 4 will show that No. 912 produced considerably more carbon dioxide than No. 894, evidence that more sugar had been utilized by the yeast. This greater consumption of sugar, coupled with the fact that a larger quantity of sugar still remained in the dough at the end of fermentation, is substantial proof that the mechanism for producing available sugar has been improved by the addition of germinated wheat.



Fig. 5. Bread Baked in the Laboratory

Baking tests on a commercial scale.—Recognizing that conclusions based upon baking data secured only in the experimental laboratory where one-loaf doughs are mixed individually might be open to criticism, it was deemed advisable to bake these flours on a commercial scale. This work was done in the baking department of Dunwoody Institute. The operations were in charge of an experienced baker who also judged the finished loaves. No

difficulty was encountered in the mixing or handling of any of the doughs. The same fermentation time was allowed in each case, but the proofing time was shortened with the germinated wheat flours. Nos. 894, 895, and 912 were proofed 85, 80, and 67 minutes, respectively. The flours from germinated wheat mixtures showed better oven spring, No. 912 excelling No. 895 in this particular. Loaf volume, texture, and more particularly grain were favorably influenced by the addition of germinated wheat. The bread from the flour milled from the 3 per cent mixture, No. 912, was classed by the scorer as exceptionally good, superior to that from the control flour. The values assigned by the scorer are given in Table XVI.

TABLE XVI

RESULTS OF BAKING TESTS AT DUNWOODY INSTITUTE OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Germinated wheat, per cent	Perfect score	Flour No.		
		894	895	912
1. Loaf volume	10	0	2	3
2. Color of crust	8	9½	9½	9¾
3. Symmetry of form	8	7¼	7¼	7
4. Evenness of bake	8	2¾	2½	1¾
5. Character of crust	8	2¼	2½	2½
6. Break and shred	8	1	2	2¾
7. Color of crumb	10	10	10	10
8. Texture	15	13	14½	14
9. Grain	10	8½	9¾	10
10. Taste	20	18½	15	16½
11. Flavor	15	14	14	14
Total bread score	100	89¼	89¾	90¾

Hydrogen-ion concentration and titratable acidity.—The influence of hydrogen-ion concentration upon the determination of diastatic activity and upon the quality of flour in general has been mentioned. Because of the importance of this characteristic, determinations were made by the procedure used for the preliminary study. At the same time the titratable acidity was determined. The results are reported in Table XVII.

The degree of acidity expressed both as pH and titratable acidity calculated as lactic acid was found to be practically the same in the flours with and without germinated wheat. It is safe to conclude, therefore, that none of the comparisons made in the study of the flours was influenced by a difference in acidity.

Effects of aging the flours.—Aging of sound, high grade bread flours is known to improve baking quality, particularly during the first four or five weeks. The results of numerous investigations

have recently been reviewed by Bailey (1925). The general conclusion seems to be that loaf volume, color, and absorption improve during the first few months, but that loaf volume may diminish after extended storage, even tho the color and absorption continue to improve. Proteolytic activity gradually decreased in storage, sprouted wheat flours retaining their activity in storage for a few years. Mangels (1924) found that there was in general an increase in diastatic activity during storage. Both common spring and durum wheat flours gave smaller loaf volumes after several months storage.

TABLE XVII

HYDROGEN-ION CONCENTRATION AS PH AND TITRATABLE ACIDITY AS LACTIC ACID IN THE FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Lab. Flour No.	Germ'nated wheat	pH	Titratable acidity as lactic acid
	Per cent		Per cent
894	0	6.12	0.13
895	2	6.13	0.13
912	3	6.14	0.14

Bailey and Johnson (1924a) reported observations of hydrogen-ion concentration during aging which show that the increase is quite rapid for the first few weeks, and then more gradual for many months. In a study of changes in hydrogen-ion concentration of wheat and its products stored under different conditions of temperature and humidity, Sharp (1924) found that the acidity increased, and, in general, more rapidly at higher temperatures and higher moisture content.

The flours used in this study were examined after 12 months storage. At the time the flours were milled, portions of each were placed in tin cans and stored in a refrigerating plant maintained at 7° to 10°C. Other portions were stored in sacks in the State Experimental Flour Mill warehouse, the temperature of which fluctuated with the temperature of the outside atmosphere since it is not artificially heated.

Effects of aging upon hydrogen-ion concentration and titratable acidity.—Hydrogen-ion concentration and titratable acidity were determined in the same manner as before, the results appearing in Table XVIII.

The hydrogen-ion concentration increased with aging, the amount of increase depending upon the conditions of storage. In

cold storage the change was about 0.08 pH and in mill storage about 0.16 pH. The germinated wheat did not seem to influence the rate of change, as the three flours showed practically the same increase. The greater increase in the samples stored in the mill is probably due to the fact that the high temperature during the summer, often 35°C. (95°F.), induced more rapid changes.

TABLE XVIII

EFFECT OF AGING UPON THE HYDROGEN-ION CONCENTRATION AND TITRATABLE ACIDITY OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Hydrogen-ion concentration			Acidity as lactic acid		
		Aged 1 month	Aged 12 months		Aged 1 month	Aged 12 months	
			Mill warehouse storage	Cold storage (7°-10°C.)		Mill warehouse storage	Cold storage (7°-10°C.)
	Per cent	pH	pH	pH	Per cent	Per cent	Per cent
894	0	6.12	5.95	6.05	0.13	0.14	0.14
895	2	6.13	5.98	6.04	0.13	0.14	0.13
912	3	6.14	5.98	6.06	0.14	0.14	0.14

The titratable acidity calculated as per cent lactic acid showed with one exception a very slight increase, not enough to be significant. The conditions of storage did not appreciably affect the changes.

Effect of aging upon diastatic activity.—Diastatic activity of the flours determined after 12 months storage is shown in Table XIX. Storage conditions were the same for the samples tested for acidity. (Table XVIII.)

TABLE XIX

EFFECTS OF AGING UPON THE DIASTATIC ACTIVITY OF THE FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Diastatic activity			
		Original	Aged 12 months		Aged 26 months
			Mill warehouse storage	Cold storage (7°-10°C.)	
	Per cent				
894	0	126	136	130	100.
895	2	204	210	208	167
912	3	241	242	243	178.

The results indicate no pronounced change in diastatic activity after one year storage, such differences as were noted being insignificant. A portion of each sample was stored in the warehouse an additional 14 months, or a total of 26 months. Diastatic activity of these samples was appreciably lower than in the fresh flour. They remained in essentially the same relation to each other, however, the flours milled from germinated wheat mixtures being higher in diastatic activity than the control flour.

Baking tests and washed gluten tests of aged flours.—In order to obtain the opinion of an experienced baker who did not know the nature of the flours, and to learn whether germinated wheat would cause deterioration in the flour on aging, two of the flours, Nos. 894 and 912, milled from the ungerminated wheat and the 3 per cent germinated wheat mixture respectively, from the mill storage, were sent to a large research laboratory without a description of their method of manufacture. Baking tests were made when the flours were 12 months old, using a formula which called for 1 per cent of yeast. No. 912 gave 3.25 per cent greater loaf volume than No. 894 and slight improvement in color. There was no evidence that the presence of the germinated wheat flour in the mixture (No. 912) had resulted in any deterioration after storage for a year. Washed gluten tests were made at the same time. No. 894 had a slightly grayish gluten, not so bright as No. 912, which was slightly yellow and also a trifle more resistant. Both samples showed a very good quality of gluten.

Five months later, or 17 months after they were milled, the same samples were again baked in the same laboratory. The absorption was increased by one per cent in both flours during the 5 months. No. 912 did not mature so fast, but exceeded No. 894 in loaf volume, this time 6.25 per cent, and showed slightly more even and velvety texture. Both flours were considered good, with preference expressed for No. 912, and no deterioration in the latter was evident from these tests.

Baking tests were also made at the State Experimental Flour Mill after the flours had been aged for 17 months. The same procedure and formula were followed as in the earlier baking trials in the same laboratory. The results given in Table XX are interesting in several respects. The different conditions of storage did not produce the same effects. The mill storage brought improvement while the cold storage was evidently responsible for deterioration, especially in point of texture. In the cold storage samples, the color was nearly the same in all three flours, but was of a creamy tint; less bleaching of the carotinoid pigments had taken place. The loaf volume of No. 895 was $2\frac{1}{2}$ per cent smaller, the others 1 to 3 per cent larger, than when the flours were fresh. The same order of volume was maintained as in the fresh flours, however, namely, larger volumes with increasing proportions of germinated wheat. No tendency toward a greater deterioration of flour milled from the germinated wheat than from the control or ungerminated wheat could be detected under either condition of storage.

TABLE XX

EFFECTS OF AGING FOR 17 MONTHS UNDER DIFFERENT CONDITIONS OF STORAGE UPON THE BAKING QUALITY OF THE FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour No.	Mill storage			Cold storage		
	894	895	912	894	895	912
Germinated wheat, per cent	0	2	3	0	2	3
Absorption, per cent	62.3	62.3	60.0	62.0	60.6	60.6
Loaf volume cc.	2200	2400	2450	1950	2120	2210
Texture, score	100	100	100	94	95	92
Color, score	99	101	101	99	99	99

The samples stored in the mill warehouse showed a marked improvement in baking quality after 17 months aging. From the standpoint of loaf volume, improvement was greater in the flours milled from germinated wheat mixtures. No. 894 increased 200 cc. with aging while Nos. 895 and 912 each increased 300 cc. The color was better in the two containing germinated wheat flour, the texture was the same. Absorption increased more with aging in the first two flours.

The baking tests warrant the conclusion that the flours milled from wheat containing 2 or 3 per cent of kernels germinated for a short time under careful control improved in baking quality with age, and after storage for nearly a year and a half showed no signs of deterioration. The flours made from the germinated wheat mixtures evidenced greater improvement than the flour from normal wheat. All three made excellent bread.

Summary and Discussion

Altho the results of each portion of this investigation have been discussed briefly as the data were reported, it appears advisable to recapitulate and summarize the results of the study as a whole.

In the first part of the work³ wheat which had been allowed to germinate for 3 days, and another portion which had germinated for 5 days were mixed with normal wheat in varying amounts and the several samples were milled on an experimental flour mill. Flour was produced which showed marked increase in diastatic activity, depending upon the quantity of germinated wheat in the mixture. The relative diastatic activity was increased from 86 in the control to 385 in the flour milled from a mixture containing 60 per cent of ungerminated wheat and 40 per cent of wheat germinated for 5 days. This large activity is probably

³ Reported in *Cereal Chemistry*, Vol. III, No. 2, pages 107 to 136.

entirely unnecessary, and further tests showed that the inclusion of sufficient sprouted wheat to effect it was undesirable. As little as 5 per cent of germinated wheat was sufficient to double the diastatic activity. Larger percentages did not increase it in the same ratio.

The length of time which the wheat germinated was found to be a most important factor. The addition of 20 per cent of wheat germinated for 3 days resulted in the same diastatic activity as 5 per cent of the wheat germinated for 5 days. While the former made a good loaf of bread, with large volume and good texture, the latter when baked gave large volume with poor texture, showing that the gas-retaining capacity of the flour had been reduced.

Flours milled from wheat mixtures containing 10 per cent or more of the wheat germinated 5 days gave unsatisfactory results when baked, and the flour from the 40 per cent mixture had lost so much gas-retaining power that the loaf was no larger than that from the 5 per cent mixture. This deterioration of the quality of the protein is ascribed principally to protease activity, and to the presence of the products of partial hydrolysis of the flour proteins.

The flours from the mixtures of wheat germinated 3 days showed progressively large volumes with increasing amounts of germinated wheat. The texture was as good as, or better than the control, up to 20 per cent of germinated wheat. The 40 per cent mixture showed that the quantity of germinated wheat was too large.

Simple nitrogenous compounds including peptids and amino compounds which result from protein hydrolysis were present in larger amounts in the flours milled from the mixtures containing wheat germinated 5 days than from those germinated 3 days. This indicates that proteolysis has proceeded further in the wheat germinated the longer period.

The results of these tests are conclusive evidence that the length of time that wheat germinates, which together with temperature and moisture content determines the length of the epicotyl exerted by the germinating kernel, deserves careful consideration in estimating the effects of germinated wheat upon normal wheat. Not only is the percentage of sprouted kernels important, but also the extent of the sprouting. This fact has a bearing upon wheat standardization and the extent to which wheat should be lowered in grade on the basis of the percentage of damaged kernels. The experiments reported here are evidence that 5 per cent of sprouted kernels from a certain lot of wheat may cause deteri-

oration in baking quality, while 20 per cent from another lot may not do so, the difference depending upon the conditions and extent of germination.

In the milling tests on a commercial scale in the Minnesota State Experimental Flour Mill, germinated wheat in amounts as small as 2 and 3 per cent had no appreciable effect upon the milling process. The diastatic activity of the straight grade flour was substantially increased, however, the increase being nearly 100 per cent with the addition of 3 per cent of germinated kernels. It should be possible to aim at a certain level of diastatic power and mill flour very close to that level, providing that the activity of the normal wheat is not too low and the character of the germinated wheat is known. In modern milling practice the protein content of the flour is determined by proper adjustment of the mill mix. These experiments indicate that the diastatic activity of flour can also be approximately adjusted.

Numerous tests were made of the flours thus milled to determine whether significant changes had taken place in characteristics other than diastatic activity. Baking tests both in the laboratory and on a small commercial scale showed definitely that the flours milled from the wheat studied, with admixtures of germinated kernels, were improved in baking value. Larger volumes were obtained, with improved texture, slightly better color of crumb, and better color of crust. The more rapid action of enzymes during fermentation, particularly in the proofing period, shortened the fermentation period.

The changes in volume of a dough and the volume of gas lost from the dough during fermentation were observed with doughs mixed from each flour. Flours milled from wheat mixtures with 2 and 3 per cent of germinated wheat produced in order more gas and retained more gas than the flour without germinated wheat. This appears to be acceptable evidence that the gas-retaining capacity of the dough was not impaired by the addition of germinated wheat.

In other respects no significant changes were observed. Protease activity studies showed slightly greater activity in the germinated wheat flours upon autolytic digestion for 46 hours, and for 6 days. Fractional nitrogen determinations which indicate hydrolysis of protein gave a slight increase with flours milled from germinated wheat mixtures. The properties of the glutenin as indicated by the viscosity of acidulated flour suspensions were practically the same in all three flours.

The inclusion of 3 per cent or less of germinated wheat caused no measurable change in hydrogen-ion concentration or titratable acidity of the flour.

Studies to determine the effect of aging were made when the flours were a year old and again after 17 and 26 months had elapsed. No appreciable change in diastatic activity was observed at the end of 12 months, but an appreciable decrease was noted at the end of the 26th month. Baking quality improved on aging these flours, the greatest improvement being observed in the flours milled from the wheat mixtures containing germinated kernels.

Conclusions

Diastatic activity of flour has been studied by numerous investigators, and the data in the literature serve to establish the importance of this property of flour in its relation to baking strength. Further evidence of this importance has been afforded by this study.

Germinated wheat may be added to wheat low in diastatic activity to supplement the deficiencies of the latter.

Germination of wheat intended for use in this connection must be conducted under controlled conditions to yield a product of the most serviceable character. Wheat sprouted for 3 days under controlled conditions proved satisfactory; that sprouted for 5 days proved unsuitable, owing, probably, to the hydrolysis of the gluten proteins and to the active proteases which such wheat contributed to the flour.

Catalase activity of the wheat proved to be a useful index of the extent of germination.

Large increases in diastatic activity of flour were effected by the addition of relatively small proportions of germinated wheat. Thus the addition of 2 or 3 per cent of germinated wheat approximately doubled the rate of sugar production in doughs made from the resulting flours. More than 3 per cent did not produce corresponding increases in diastatic activity. It appears probable that from 3 to 5 per cent will give optimum results in mixture with wheats which alone yield flours with a diastatic activity ranging around 100 units. Control of the diastatic activity of flour normally low in that property can be effected within ordinary limits by graduated additions of germinated kernels to the wheat used in the production of the flour.

Milling processes are not interfered with and milling yields are unaffected when 2 or 3 per cent of wheat kernels germinated for 3 days are present in the wheat mixture.

Baking strength of flour milled from wheat low in diastatic activity was increased when 3 per cent of sprouted kernels was added to the wheat. The increased strength was registered in terms of a decidedly increased gas production, a small increase in gas retention, increased loaf volume, and superior grain and texture. Time for proofing or raising the dough in the pan was reduced; color of the crumb appeared whiter, and the crust of the loaves was browner and more pleasing in appearance when the mixture contained germinated wheat.

Protein quality was seemingly unaffected by the addition of 2 or 3 per cent of wheat which had been germinated for 3 days. Protease activity was not increased appreciably, so far as could be detected.

Aging of flour milled from wheat mixtures containing up to 3 per cent of germinated kernels proceeded normally. Baking strength of such flour under ordinary conditions of storage increased somewhat with the lapse of time. Examination of the flour after 26 months of storage showed it to be as sound as, and superior in baking strength to flour milled at the same time from ungerminated wheat.

Literature Cited

Bailey, C. H.

1916. A method for the determination of the strength and baking qualities of wheat flours. *J. Ind. Eng. Chem.*, Vol. 8, pp. 53-57.

1922. Report of operations, State Testing Mill. Crop season of 1921-22. *Minn. State Dept. Agr. Bull.* 23.

1925. The chemistry of wheat flour. Chemical Catalog Company, New York.

— and Johnson, A. H.

- 1924a. Studies of wheat flour grades. IV. Changes in hydrogen-ion concentration and electrolytic resistance of water extracts of natural and chlorine-treated flours in storage. *Cereal Chem.* Vol. I, pp. 133-137.

- 1924b. Carbon dioxide diffusion ratio of wheat flour dough as a measure of fermentation period. *Cereal Chem.* Vol. I, pp. 293-304.

Gortner, R. A.

1924. Viscosity as a measure of gluten quality. *Cereal Chem.* Vol. I, pp. 75-81.

Mangels, C. F.

1924. Effect of storage on baking quality of common and durum wheats. *Cereal Chem.* Vol. I, pp. 168-178.

Sharp, P. F.

1924. Wheat and flour studies. II. Aging. I. The change in hydrogen-ion concentration of wheat and mill products with age. *Cereal Chem.* Vol. I, pp. 117-132.

Sörensen, S. P. L.

1907. Etudes Enzymatiques. *Compt. rend. Lab. Carlsberg*, Vol. 7, pp. 1-57.

A RAPID METHOD FOR THE COLORIMETRIC DETERMINATION OF HYDROGEN-ION CONCENTRATION OF CRACKERS

By R. T. BOHN AND R. J. MARTZ,

Perfection Biscuit Company, Fort Wayne, Ind.

(Received for publication February 26, 1926)

The electrometric and colorimetric methods for the determination of hydrogen ion in crackers have recently been employed by Johnson and Bailey (1924). The electrometric determination as described by them requires elaborate and expensive electrical apparatus and a chemist skilled in the handling of it. The colorimetric method has many advantages. There has been great need for a method which would not require such expensive equipment and which could be used by the cracker baker as an aid in judging the degree of acidity or alkalinity of the crackers made under his supervision. Many qualities in a cracker enable the practical baker to hazard a good guess as to the alkalinity of his crackers, from which he can judge if he has added the correct amount of soda to his doughs, but the tendency has been to acquire as much scientific training as possible, and this method was developed to give him an exact check on degree of alkalinity of crackers. It also offers a possibility of the rapid determination of hydrogen-ion concentration in crackers to the chemist in a plant not equipped with a potentiometer outfit.

The use of indicators for the determination of hydrogen-ion concentration has been described by Clark (1922). These indicators are now on the market in a high degree of purity. The indicators in the concentration specified by Clark and Lubs (Clark, 1922) were used throughout this work. The colors obtained with crackers, using the method described later, were compared with the color chart in Clark's book; but as this chart has its limitations, a series of color standards was used. These were made by mixing 10 ml. of Clark and Lubs buffer solution with 0.5 ml. of indicator solution, the resulting colors being the specific shades exhibited by that indicator at the pH values of the buffer mixtures used. The LaMotte h-ion comparator set containing these color standards in sealed ampoules may be secured from the LaMotte Chemical Products Company and are very stable if kept in a dark place when not in use.

Johnson and Bailey (1924) used the colorimetric determination in conjunction with their work on pH of crackers with the potentiometer and state that "observation made by colorimetric method agreed with the more accurate determination by the potentiometer method, within the limits of accuracy anticipated in such determinations," but give no results. The authors investigated this and have found that the colorimetric method can be used to give very close checks on the electrometric determination. The method is as follows:

The crackers were ground in a mortar to a fine meal. Ten grams of this meal was digested with 100 cc. of distilled water at room temperature and after standing five to ten minutes, filtered through cheese-cloth. The pH of the resulting suspension was determined, using phenol red in alcoholic solution, comparing the color so obtained with color of standard tubes for this indicator, using the LaMotte h-ion comparator outfit. The pH was also determined electrometrically. These data are recorded in Table I.

TABLE I
COMPARISON OF ELECTROMETRIC AND COLORIMETRIC METHODS

Sample No.	Electrometric	Colorimetric
	pH	pH
1	7.2	7.3
2	6.9	6.9
3	7.5	7.5
4	6.8	6.8

A skilled operator can obtain very close checks, especially on pH within the range of phenol red and cresol red. The same results can be obtained using a water solution of these indicators.

The colorimetric method of digesting the cracker with water and using a comparator outfit can be made a practical method for the cracker baker, but it requires the use of distilled water, the grinding of the cracker, and filtration, and at its best requires ten minutes. By using such a method in the plant, very good checks could be obtained on electrometric titrations, using the same concentration of cracker meal. However, this does not represent the true pH of the cracker but of the cracker meal in a certain dilution. Johnson and Bailey's figures indicate this. They found very little difference between pH of cracker meal suspension of 20% and 10% concentration but 0.3 less on 5% concentration, indicating, as is to be expected, a greater ionization in the more dilute concentration. This is, of course, not noticeable in crackers which are neutral in reaction. The data in Table II, determined colorimetrically, show this difference.

TABLE II
RELATION OF CONCENTRATION OF CRACKER MEAL TO pH OF EXTRACT DETERMINED
COLORIMETRICALLY

Grams of cracker meal per 100 ml. water	Alkaline crackers		Neutral crackers
	pH	pH	pH
1	7.4	6.9	6.8
5	8.0	7.6	6.9
10	8.4	7.6	6.9
20	8.7	7.8	6.9

As is to be expected, cracker meal which is close to neutral in the different concentrations shows very little difference in pH. There is, however, a change of pH of cracker meal with concentration when there is a decided alkalinity. This also is to be expected.

That the "salt error" of the indicator plays very little, if any part, in this increase of pH with concentration, is shown in Table III, which was obtained on crackers containing no salt. The same increasing values with concentration is shown.

TABLE III
RELATION OF CONCENTRATION OF CRACKER MEAL WITHOUT SALT TO pH

Grams of cracker meal per 100 ml. water	pH
1	7.6
5	8.2
10	8.6
20	8.7

With a view to making the colorimetric test shorter and at the same time accurate, the authors have carefully studied the results obtained by placing a drop of indicator on the broken edge of a cracker and comparing the colors thus obtained with the series of color standards for these indicators. It was found that none of the indicators gave a distinct coloration when used in alcoholic solution. In this case, to obtain a coloration which was known to be in accordance with the degree of alkalinity of the cracker, it was necessary to moisten the cracker with a drop of distilled water. Water solutions of the indicators can be used directly. There is a tendency, however, to secure higher results using a water solution of the indicator, altho they are nearer the true pH of the cracker. When used alone, the indicator is quickly absorbed. Better results are obtained by moistening the broken edge of the cracker with distilled water and then testing with a suitable indicator, in either alcohol or water solution.

The method.—Several drops of distilled water are distributed across the freshly broken surface of the cracker, then several drops of water or alcoholic solution of phenol red are placed on this, and

the color that results is compared with the colors of standard tubes for this indicator. If the color is near the limit of the range of the indicator, cresol red is used. Comparison was made with results obtained by the electrometric and colorimetric methods, using 10 grams of cracker meal to 100 ml. of water. The results are as follows:

TABLE IV
COMPARISON OF pH OF CRACKERS USING THE PROPOSED RAPID METHOD

Sample	Electrometric pH	Colorimetric pH	Rapid colorimetric pH
1	7.2	7.3	7.7
2	6.9	6.9	7.0
3	7.5	7.6	8.2
4	6.8	6.8	6.8
5	7.7	7.7	8.3
6	8.0	8.2	8.6
7	8.2	8.2	8.6

It will be noticed that the pH on crackers using the rapid colorimetric method is the same when pH is around 6.9, and is higher when pH is over 7.2. These results are to be expected from the discussion just above. The pH obtained on crackers by the rapid test of the authors is nearer the true value, hence higher in alkaline crackers than figures obtained using an empirical concentration and determining pH electrometrically and colorimetrically.

It will be noticed on using this method that a thin sheet at the top and bottom of the cracker is usually yellow. This is due to the dusting flour used. In grinding the whole cracker to a meal, the resulting sample includes, therefore, a portion of the cracker which is not representative of the true condition of the cracker. This again accounts for higher pH values of the rapid method.

It is usual factory practice to run crackers on the alkaline side. Johnson and Bailey (1924), out of 60 samples examined, showed only 15 with pH less than 6.9, and of these only 10 were below the limit of range of phenol red; 15 were above the range. So for general work, phenol red can be used. For pH above range of this indicator, cresol red is suggested in the method described above, in preference to thymol blue. When pH is around 7.9, cresol red should be used to check the pH obtained with phenol red.

The writers have prepared a wooden block which contains the standard tubes with a white background and sufficient space between them to insert a cracker so as to secure good color comparison. This has proved a great help. It is not necessary to bother with individual tubes, they are all visible and protected from breakage. This method is in daily use in the Perfection Bis-

cuit Company, and it has been found to check well with the regular colorimetric and electrometric methods. It gives higher pH values when excess soda is large, but these can be standardized for each plant. In the hands of an unskilled operator, pH can easily be determined to within 0.2.

There are but a few precautions to be noted in using this method; if using an alcoholic solution of the indicator, the cracker must first be moistened with distilled water. This is important. If the pH value is near the limit of range of the indicator used, check it by using the next indicator in the series. Do not select a cracker which is overbaked, as sometimes happens on the edge of a peel or pan; an overbaked cracker will show lower alkalinity.

In the hands of the cracker baker, this method is a valuable asset. He soon learns the pH corresponding to the color he obtains in his test without consulting the standard tubes. It is quick and accurate. By keeping dough temperatures constant and fermentation conditions the same, he can vary the soda until he secures the desired alkalinity or acidity in the cracker. The variation in soda requirements in individual doughs is usually not very large and a moderate excess of soda above that actually required to secure the desired alkalinity or acidity in the crackers does no harm.

The writers at this time wish to express appreciation of the interest and assistance of Pearl Brown in bringing this work to a successful conclusion.

Literature Cited

- Clark, W. M.
1922. The determination of hydrogen ions. Williams and Wilkins Co., Baltimore, Md.
- Johnson, A. H. and Bailey, C. H.
1924. A physico-chemical study of cracker dough fermentation. Cereal Chem. Vol. I, pp. 327-410.

THE GASOLINE COLOR VALUE OF SEVERAL CLASSES OF WHEAT

By D. A. COLEMAN AND ALFRED CHRISTIE

Grain Investigations, Bureau of Agricultural Economics,
United States Department of Agriculture

(Received for publication March 22, 1926)

Color is of special importance in the marketing of some classes of wheat. In the bread wheats, lack of color is desired; whereas in the durum wheats, a rich color is preferred. Certain varieties of durum wheat are lacking in color, and others yield semolinas which produce pastes of a decided gray or brownish color. For this reason, durum wheat buyers endeavor to select their purchases with care, having in mind the securing of a desirable and uniform color in the manufactured products.

With a view to obtaining some information concerning the magnitude of the principal coloring matter in wheats, particularly in the durum varieties, tests were made relative to the pigments soluble in gasoline, as they are to be found in the straight grade flours milled from the various classes and varieties of wheat. As a means of extracting these pigments the method described by the authors¹ in another paper was used.

The durum wheat flours tested were obtained from wheats grown by the Office of Cereal Investigations, Bureau of Plant Industry, during the crop years 1919 to 1924, inclusive. For comparison, flours milled from certain samples of the other commercial classes of wheat were studied and the data are here presented.

Data resulting from a study of the gasoline color value of the flours milled from the durum wheats are given in Table I. For clarity the value 1 in Table I is equivalent in color intensity to a .005 per cent solution of potassium chromate.

A study of Table I, in which the varieties are arranged in the descending order of their average gasoline color value, shows that they may be divided into two main groups. One group is composed of the varieties Arnautka, Kahla, Kubanka, and Mindum; the other group of Acme, Pentad, and Monad. The varieties in the latter group contain less of the gasoline soluble pigments than any of the other varieties, the rust resistant wheats Acme and Monad, being particularly low in such pigments. The greatest amount of pigment was found in certain strains of Arnautka and Kubanka.

¹ A rapid method for determining the gasoline color value of flour and wheat. By D. A. Coleman and Alfred Christie, *Cereal Chem.*, Vol. III, No. 2, p. 84, 1926.

In Figures 1 and 2 are given in graphic form the statistics obtained from the durum wheat flours tested.

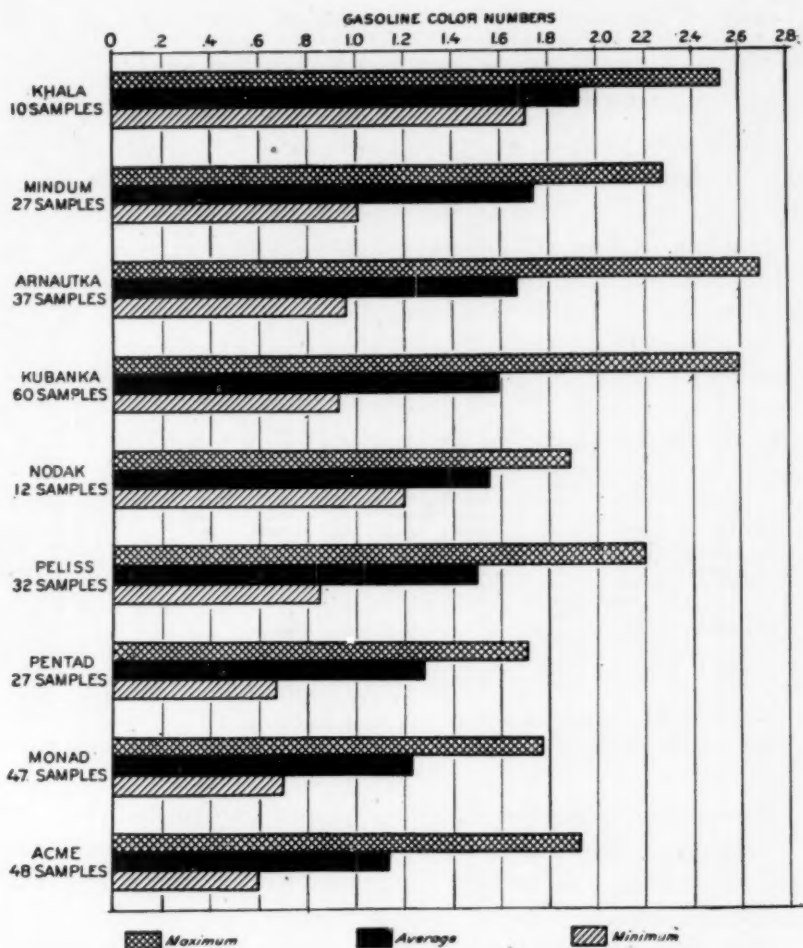


Fig. 1. The Maximum Average and Minimum Gasoline Color Numbers Nine Important Varieties of Durum Wheat, 1919-1924.

Gasoline Color Value of Flour Milled from Other Commercial Classes of Wheats

The gasoline color values of the straight flours from several other classes of wheats were determined and are presented in Table II. In this table it will be seen that the average color of the spring wheat flour tested was 1.39; of the hard red winter wheat flour, 1.69; of the soft red winter wheat flour, 1.67; and that of the hard white wheat flour,

1.41. The soft white wheat flours had the lowest color of all the classes tested. It should be pointed out that flours from certain samples of all the classes of wheats studied had just as high gasoline color value as did many of the durum wheat flours, which as a rule are high in color pigments.

Gasoline Color Test Applied to Wheat

Tests were made on ground wheat to meet the objection that color values obtained from a study of the flour might not reflect the inherent color in the wheat because of the impossibility of always extracting the same amount of flour from the wheat. Then, too, there was the practical consideration that much time and labor would be saved if the determination could be applied with success to the ground wheat.

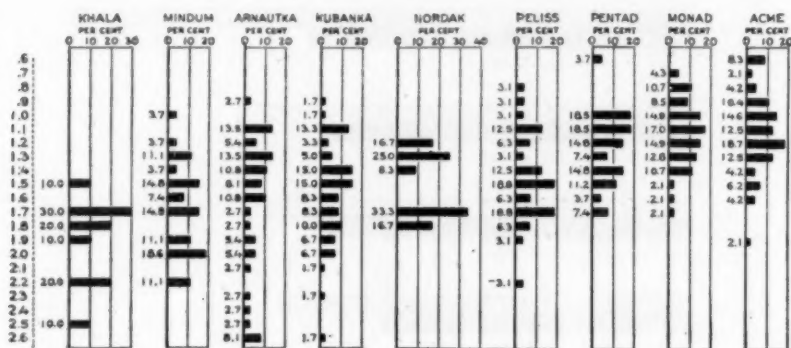


Fig. 2. Gasoline Color Value for Each Variety of Durum Wheat

They are arranged to show the percentages of the samples which contained the indicated color value.

Before proceeding far, difficulties arose regarding the preparation of the sample. After considerable study it was found that good results could be obtained if the sample was ground so that at least 75 per cent of it passed through a No. 50 grits gauze sieve.

Results obtained with whole wheat samples are given in Table III. They confirm the earlier observations made on straight flours from the same classes of wheats (see Table II), namely, that wheats of other commercial classes contain as much pigment extractable in gasoline as do some of the durum varieties.

TABLE III
GASOLINE COLOR VALUE OF SEVERAL CLASSES OF AMERICAN WHEATS

No. of samples	Class of wheat	Gasoline color value		
		Average	Maximum	Minimum
26	Durum	1.39	2.07	.80
8	Hard red spring	1.38	1.73	.75
22	Hard red winter	1.68	2.29	1.28
25	Soft red winter	1.17	2.00	.64
28	White	1.60	2.40	.93

TABLE I
NUMBER OF SAMPLES OF VARIETIES OF DURUM WHEAT FALLING WITHIN THE VARIOUS RANGES OF GASOLINE COLOR NUMBER

Varieties of durum wheat	Gasoline color number																				Average gasoline Total color	
	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5		2.6
Kahla										1		3	2	1			2			1		10
Mindum					1							4		3	5		3					27
Arnautka				1		5	2	5	4	3	4	1	1	2	2	1		1	1	1	3	37
Kubanka				1	1	8	2	3	9	9	5	5	6	4	4	1		1			1	60
Nordak							2	3	1			4	2									12
Peliss			1	1	1	4	2	1	4	6	2	6	2	1			1					32
Pentad	1			5	5	4	2	4	3	1	1	2										27
Monad		2	5	4	7	8	7	6	5	1	1	1										47
Acme	4	1	2	5	7	6	9	6	2	3	2			1								48
																						1.16

TABLE II
GASOLINE COLOR VALUES OF WHEAT FLOUR MILLED FROM OTHER CLASSES OF WHEAT

No. of samples	Classes of wheat					Common white
	Hard red spring	Hard red winter	Soft red winter	Hard white		
Average	18	22	10	8	5	
Maximum	1.39*	1.89	1.67	1.41	1.13	
Minimum	1.55	2.06	1.87	1.95	1.22	
Range	1.25	1.44	1.80	1.32	1.00	
	.30	.62	.27	.63	.22	

* The value 1 is equal in color intensity to a .005% potassium chromate solution.

**Correlation Between Gasoline Color Value of Flour and Protein
Content of 194 Samples of Durum Wheat Flour
(Straight Grade)**

Studies were also made comparing the crude protein content of the flour and its gasoline color value for the purpose of noting whether color is associated with protein content. The data are shown in Table IV, where it can be easily seen that there is no significant relation between the protein content of the flour and the amount of pigment soluble in gasoline.

TABLE IV
CORRELATION BETWEEN GASOLINE COLOR VALUE AND CRUDE PROTEIN CONTENT OF 194 SAMPLES
OF DURUM FLOURS

% Protein	Gasoline color range											Total
	.6 - .799	.8 - .899	1.0 - 1.199	1.2 - 1.399	1.4 - 1.599	1.6 - 1.799	1.8 - 1.999	2.0 - 2.199	2.2 - 2.399	2.4 - 2.599	2.6 - 2.799	
10.5 to 11.49				1		2						3
11.5 to 12.49	1				2	1						4
12.5 to 13.49			3	2	2	4	2					13
13.5 to 14.49	1	5	7	6	5	3	3					30
14.5 to 15.49		2	9	4	7	1	1	2				26
15.5 to 16.49		2	7	6	6	6		2			1	30
16.5 to 17.49			2	3	8		2			1		16
17.5 to 18.49	1	3	4	4	2	3	2		2			21
18.5 to 19.49	2	1	7	6	7	1	1		1			26
19.5 to 20.49	3	3	4	3	4			1		1		19
20.5 to 21.49		1	2	1	1	1						6
Totals	8	17	45	36	44	22	11	5	3	2	1	194

Coefficient of correlation (r) = .091 \pm .048

**Correlation Between Gasoline Color Value of Flour and the Ash
Content of 194 Samples of Durum Wheat Flour,
(Straight Grade)**

It is reasonable to suppose that low flour color will be associated with low ash content; indeed ash content is sometimes used as a measure of flour color. For every increase or decrease in unit of ash, a proportionate amount is subtracted or added to the color value. For example, a standard patent of 0.48 per cent ash may be used for the 100 per cent value. Allowing an increase of one for every decrease of two in the hundredth place of ash, the color of a 0.38 per cent ash would read 105 per cent, one with a 0.54 per cent ash would have a color value of 97 per cent, and one with a 0.72 per cent ash would be rated 88 per cent.

In order to see how the percentage of ash correlated with the gasoline color value of a number of straight grade durum wheat flours, ash determinations were made along with the gasoline color value of the same sample and the relationship compared. These data are given

in Table V and bring out that the expected positive relationship between the ash content of the flour and the pigments soluble in gasoline did not materialize.

TABLE V
CORRELATION BETWEEN GASOLINE COLOR VALUE AND ASH CONTENT OF 194 SAMPLES OF DURUM FLOUR (STRAIGHT GRADE)

% Ash	Gasoline color range											Total
	.6 - .799	.8 - .999	1.0 - 1.199	1.2 - 1.399	1.4 - 1.599	1.6 - 1.799	1.8 - 1.999	2.0 - 2.199	2.2 - 2.399	2.4 - 2.599	2.6 - 2.799	
.45 to .549		1			1							2
.55 to .649			5		1	1						7
.65 to .749		2	6	3	1	2	3	1				18
.75 to .849	3	4	13	7	12	6	1		2	1		49
.85 to .949	2	4	12	14	12	2	4	1				51
.95 to 1.049	1	3	6	7	5	5	2	1		1		31
1.05 to 1.149	1		1	2	7	3	1	1	1		1	18
1.15 to 1.249	1	1	2	1	1	3		1				10
1.25 to 1.349		2		2	3							7
1.35 to 1.449					1							1
Totals	8	17	45	36	44	22	11	5	3	2	1	194

Coefficient of correlation (r) = $.101 \pm .048$

Summary

A study was made of the pigments found in wheat and wheat flour which are soluble in gasoline. Quantitatively, these pigments vary greatly. The largest amount, 2.40, was found in a sample of hard white wheat, the lowest, .64, in a sample of soft red winter wheat.

On the average, the wheats of the hard red winter class contained the largest quantity of gasoline-soluble pigments, the next greatest amount being found in the common white class of wheat. It was surprising to note that the durum wheat averaged lower in gasoline soluble pigments than the wheats of the classes mentioned, as durum wheats are usually associated with high color.

Certain varieties of durum wheat are consistently lower in gasoline-soluble pigments than others. The varieties Arnautka, Kubanka, Kahla, and Mindum contain large quantities of these pigments, whereas the varieties Acme, Monad, and Pentad contain low amounts of these pigments.

No relationship was found between the protein and ash content of durum wheat flour (straight grade) and the gasoline soluble pigments.

The gasoline color test can be applied to the wheats as successfully as to the flours.

A NEW FACTOR FOR CONVERTING THE PERCENTAGE OF NITROGEN IN WHEAT INTO THAT OF PROTEIN

By D. BREESE JONES

Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington

(Received for publication April 1, 1926)

The percentage of crude protein in most foods and feeding stuffs is obtained, as is well known, by determining nitrogen according to the Kjeldahl method and multiplying the result by the factor 6.25. This factor is based on the assumption that the average percentage of nitrogen in pure proteins lies around 16 per cent. The nitrogen content of proteins, however, varies within a rather wide range. Some proteins have been reported as having as little as 12 per cent of nitrogen; others contain as much as 19 per cent. It is evident that when dealing with a pure protein or with a mixture of proteins of known composition, a factor can be used which when multiplied by the nitrogen content of the substance in question will give the exact percentage of protein. However, when dealing with materials containing complex mixtures of various proteins in unknown proportions, no factor can be derived that will exactly correspond to the nitrogen percentage of the total proteins present.

The factor 6.25 usually employed is based on the nitrogen content of only a few well known proteins, chiefly of animal origin, which contain about 16 per cent of nitrogen. The average percentage of nitrogen in forty-nine different proteins, mostly of vegetable origin, listed by Plimmer (1917) is 17.1 per cent. This corresponds to the factor 5.84. In calculating the percentage of protein in most vegetable mixtures, this factor would therefore give the protein content more accurately than the usual factor, 6.25. In dealing with substances the protein content of which is fairly well known, both as to the composition of the proteins and the relative proportions in which they occur in the substance, special factors are used. Thus, the protein content of milk is obtained by multiplying the nitrogen by the factor 6.38. This factor is used because the principal proteins of milk—casein and lactalbumin—contain about 15.7 per cent of nitrogen. The factor generally used for wheat flour and for the whole wheat kernel, 5.7, is based on the nitrogen content of the two principal proteins in flour—gliadin and glutenin.

However, millers know that in producing flour from wheat of a given protein content they get flour with a protein content about 1 per cent lower than that of the wheat. This has been assumed to be due to the fact that bran contains a higher percentage of protein than the endosperm. Recent work done in the Bureau of Chemistry on the proteins of bran has shown that this is only one factor. The other cause of the lower protein content of the flour as compared to that of the wheat from which it is made is that the proteins of bran have not the same nitrogen percentage as the proteins of flour. Two of them have a lower percentage. The nitrogen of bran represents approximately 22 per cent of the total nitrogen of wheat. Working with a commercial wheat bran from which nearly all the adhering parts of the starchy endosperm had been removed by special treatment, Jones and Gersdorff (1923) isolated three proteins—an albumin, a globulin, and a prolamins—which were obtained in percentages of 2.87, 2.35, and 5.35 respectively, and which amounted collectively to 10.57 per cent of the bran. These proteins have been extensively studied with regard both to their chemical composition (1925) and properties and to their nutritive value.

The results of these studies have raised the question whether or not a factor based not only on the nitrogen content of gliadin and glutenin, but also taking into account the proteins of the bran and the embryo, would give more accurately the protein content of wheat than the customary factor 5.7. Special interest lends itself to this matter in view of the current practice of buying wheat upon analysis and determination of its protein content. We have, therefore, calculated the factors for converting the percentages of nitrogen in the endosperm, embryo, and bran into terms of protein. On the basis of the nitrogen percentages in these different parts of the seed and of their relative distribution in the wheat kernel, a factor has been obtained for the conversion of the nitrogen of the whole kernel into terms of protein.

The Endosperm

The proteins of the endosperm consist chiefly of gliadin and glutenin, which are present in wheat in approximately equal quantities. Altho traces of a globulin and albumin have also been isolated from flour, it is questionable whether or not these small quantities found in the best flour may be due to the presence of small quantities of embryo that escape separation in the milling process. At any rate, the quantities are too small significantly to

affect the results when considering the conversion factors for the endosperm nitrogen. Gliadin contains about 17.6 per cent of nitrogen, and glutenin about 17.5. The endosperm proteins collectively, therefore, contain 17.55 per cent of nitrogen, which, divided into 100, gives the conversion factor 5.698, or 5.7, which is the factor generally used for the conversion of nitrogen into protein percentage, not only in flour, but also in whole wheat.

The Bran

The proteins of bran have been shown to consist essentially of an albumin, a globulin, and a prolamins. The proportions in which these proteins have been isolated from bran and the percentages of nitrogen which they contain are as follows:

Protein	Percentage of nitrogen	Amount isolated for 100 gm. bran
Albumin	15.4	2.87
Globulin	17.7	2.35
Prolamin	15.3	5.85
Total		10.87

On the basis of these data, the bran proteins collectively contain 15.86 per cent of nitrogen, corresponding to the conversion factor 6.31.

The Embryo

Osborne (1924) obtained from wheat embryo 10 per cent of albumin, 5 per cent of globulin, and about 3 per cent of proteose, which contain 16.8, 18.3, and 17.0 per cent of nitrogen, respectively. Calculated on the basis of these figures, the embryo proteins as a whole contain 17.24 per cent of nitrogen, corresponding to the conversion factor 5.80.

The Whole Wheat Kernel

In their classic work on the nutritive value of the wheat kernel and its milling products, Osborne and Mendel (1919) have estimated the percentages of the total protein of the wheat kernel which is contained in the endosperm, bran, and embryo.

Part of kernel	Percentage of total protein of kernel
Endosperm	73.3
Bran	22.3
Embryo	4.4

These figures are based on approximate proportions of the above named parts in the average moisture-free wheat kernel containing 2.2 per cent of nitrogen. The authors state "the relative proportion of these parts varies somewhat in different samples of wheat, but in general we believe that these figures fairly represent the average."

Taking into account the foregoing proportions of the parts in the seed, the relative quantities of the different proteins which have been isolated from these parts and the percentages of nitrogen in the proteins, the conversion factor for the nitrogen of the whole wheat kernel may be calculated in the following manner:

Total nitrogen in 73.3 gm. of endosperm protein:
 $73.3 \times 0.1755 \dots\dots\dots 12.864 \text{ gm.}$

Total nitrogen in 22.3 gm. of bran proteins:

$2.87 \times 22.3 \times 0.154$
 $\frac{10.57}{2.35 \times 22.3 \times 0.177} = 0.9325 \text{ gm. albumin nitrogen.}$

$\frac{10.57}{5.35 \times 22.3 \times 0.153} = 0.8775 \text{ gm. globulin nitrogen.}$

$\frac{10.57}{10.57} = 1.7269 \text{ gm. prolamin nitrogen.}$

Total $\dots\dots\dots 3.537 \text{ gm.}$

Total nitrogen in 4.4 gm. of embryo proteins:

$10 \times 4.4 \times 0.168$
 $\frac{18}{5 \times 4.4 \times 0.183} = 0.411 \text{ gm. albumin nitrogen.}$

$\frac{18}{3 \times 4.4 \times 0.17} = 0.224 \text{ gm. globulin nitrogen.}$

$\frac{18}{18} = 0.125 \text{ proteose nitrogen.}$

Total $\dots\dots\dots 0.760 \text{ gm.}$

Total nitrogen in 100 gm. of total wheat proteins. $\dots\dots\dots 17.161$

$100 \div 17.161 = 5.83 \text{ conversion factor.}$

The conversion factor 5.83 thus obtained is based partly on the relative proportions in which the proteins have been isolated from the different parts of the seed. The quantities actually isolated, however, probably do not represent all of these proteins which are present, especially in the case of the embryo and the bran. It is seldom that the proteins in a naturally occurring plant or animal product can be completely extracted. Furthermore, there are always more or less unavoidable losses involved in the separation and isolation of the proteins after they have been extracted from the original material which contained them. Such losses are usually, however, rather uniformly distributed among the different proteins of the seed and would therefore not significantly affect the results when calculating the conversion factor. Altho the new factor here presented for the conversion of the percentage of nitrogen of wheat into protein does not differ greatly from the factor in general use, it is believed that it will give more accurately the protein content of wheat than the customary factor 5.7.

Summary

In the light of results recently obtained from a study of the proteins of wheat bran, a new factor for the conversion of the percentage of nitrogen in wheat into terms of protein has been calculated. The conversion factors for the nitrogen in the three parts of the kernel are: bran, 6.31; endosperm, 5.70; embryo, 5.80. By basing the calculation on the percentages of nitrogen in the individual proteins of the endosperm, embryo, and bran, and on the relative proportions in which these proteins are present, the conversion factor 5.83 is obtained for the nitrogen of the whole kernel, instead of 5.7, the factor generally used.

Literature Cited

- Jones, D. B., and Gersdorff, C. E. F.
 1923. Proteins of wheat bran. I. Isolation and elementary analyses of a globulin, albumin, and prolamin. *J. Biol. Chem.*, Vol 58, pp. 117-131.
1925. Proteins of wheat bran. II. Distribution of nitrogen, percentages of amino acids and of free amino nitrogen: A comparison of the bran proteins with the corresponding proteins of wheat endosperm and embryo. *J. Biol. Chem.*, Vol. 64, pp. 241-251.
- Plimmer, R. H. A.
 1917. The chemical constitution of the proteins. Pt. 1, p. 131. London.
- Osborne, T. B.
 1924. The vegetable proteins, 2nd Ed. p. 10.
 and Mendel, L. B.
 1919. The nutritive value of the wheat kernel and its milling products. *J. Biol. Chem.*, Vol. 37, pp. 557-601.

EFFECT OF MONO CALCIUM PHOSPHATE UPON THE VISCOSITY OF ACIDULATED FLOUR-IN-WATER SUSPENSIONS

By LAWRENCE EARLENBAUGH

A Correction

Table III, page 106, March, 1926, should read as follows:

TABLE III
 ASH CONTENT OF NATURAL AND PHOSPHATED FLOUR AFTER WASHING THE FLOUR WITH WATER AND WITH PHOSPHATE SOLUTION

Flour	Washed with water		Washed with phosphate solution
	Ash of natural flour	Ash of phosphated flour	Ash of natural flour
A	0.286	0.381	0.366
B	0.260	0.352	0.345
C	0.204	0.243	0.240

BOOK REVIEW

Percy A. Amos, *Processes of Flour Manufacture*, New Edition revised by James Grant. Published by Longmans Green and Company, New York, 1925.

The first edition of this text book appeared in 1912 and constituted one of the earliest comprehensive treatises on roller milling written in the English language. While it detailed the practices of British flour mills, the discussion of the fundamentals of milling operations was of interest to American millers as well. This new edition has been prepared for publication by Professor James Grant, colleague of the late Percy A. Amos, in the Manchester School of Technology (England).

The text is organized in several chapters which cover milling conditions in England, past and present; history of flour milling; the wheat berry; the world's wheats and wheat lands; parcels for wheat mixtures; mill planning and construction; grain intake and stock handling; preliminary cleaning of wheat; wheat storage; screening, grading, and dressing mediums; dry cleaning of wheat; wheat washing and whizzing; wheat drying and conditioning; handling of screenings; wheat blends and mixtures; gradual reduction system; break system; scalping and grading; sifters and plansifters; flow sheet design; purification; reduction; flour dressing; flour offals; mill staffing and management; rules, regulations, and accidents; power and power transmission; fire risks and safeguards; capacities and speeds of machines; general data; general science applied to milling; examination questions, and a bibliography. The book contains over 120 illustrations, chiefly of cleaning and milling machines, and diagrams of flows or programs.

This latest edition includes some new material which did not appear in the first edition. Disc separators for cleaning wheat are described in Chapter XII; there are several additional illustrations of roller mills and sifters; and a section has been added which presents certain applications of general science to flour milling. The relative dearth of new material appears, however, to be a sad commentary on the progress which the industry has made during the thirteen years between 1912 and 1925.

Surprisingly little space has been devoted to the discussion of air conditioning and the effect of varying atmospheric conditions upon the properties of flour and other mill products. The discussion of wheat inspection and grading in the United States (p. 17)

displays a lack of familiarity with the Federal wheat standards. It is unfortunate that the extensive data compiled and published by Shollenberger and his colleagues were not utilized in developing the consideration of the milling properties of American classes of wheat. Moreover, it seems unfortunate to illustrate the moisture tester developed by Brown and Duvel over the name of a manufacturer, even though the accompanying text properly refers to the inventors of this important device.

Artificial bleaching is discussed briefly in the chapter on flour, but this phase of flour treatment is incomplete, as practically no reference is made to the newer processes involving the use of nitrogen trichloride, and benzoyl peroxide, while only casual reference is made to the use of chlorine. The discussion of flour improvers is surprisingly brief, in view of the English origin of this manuscript, the section in question covering only a scant half page of the text.

One cannot but be impressed, however, with the care displayed in organizing the sections in which the mechanical processes and machines are discussed. The terse yet comprehensive manner in which certain mechanical operations in milling are discussed for the student is pleasing. Amos set a high standard as a teacher of this difficult subject involving an industry in which practice advanced faster than the laboratory demonstration of fundamental principles.

C. H. BAILEY.